EFFECTS OF PETROLEUM CONTAMINATED WATERWAYS ON SPAWNING MIGRATION OF PACIFIC SALMON PHASE I. LABORATORY STUDIES

by

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SUMMARY

Because oil exposure has been shown to cause chemosensory disruption and behavioral changes in other marine organisms, and because salmon depend on chemosensory detection of chemical cues during the coastal as well as freshwater phase of their spawning migration, there is concern that oil spilled into the path of migrating salmon may disrupt their spawning migration. The general objective of this project was to determine whether exposure to oil-contaminated water would disrupt the ability of adult Pacific salmon to migrate. Phase 1 of the project consisted of laboratory studies of the effects of oil on salmon chemosensory function. Phase II will consist of field experiments on the effects of petroleum-contaminated waterways on salmon migration. The findings of Phase I are to be used in designing the fieldwork of Phase II. After providing a background on the spawning migration of salmon and the potential effects of oil spills, this report presents the findings to date in the Phase I laboratory work.

Electro-olfactogram (EOG) experiments were used to determine the concentration at which adult male coho salmon detect water-soluble fraction (WSF) of Alaska North Slope (ANS) crude oil. With different protocols of stimulation and experimental treatment of the fish, EOG techniques were also used to examine changes in chemosensory function of adult coho salmon at higher concentrations of the WSF of ANS crude oil and the effects of short-term exposure of adult coho salmon to the WSF of ANS crude oil on detection of an amino acid mixture. There are no techniques for directly measuring motivational state or any adequate physiological assays of early spawning condition. However, examination of how motivation might be influencing the various responses of salmon to petroleum hydrocarbons was attempted through post hoc correlations of olfactory responses observed on a particular date with the hormonal status of that fish measured using radioimmunoassay (RIA) techniques.

Using EOG techniques, adult coho salmon, Oncorhynchus kisutch, were found to have an estimated detection threshold for the WSF of ANS crude oil on the order of IO^{-10*1} mg/l WSF or about 10^{-7} ppb. At WSF concentrations above 10^{-4} mg/l, the chemosensory response to WSF was degraded but not irreversibly. After short presentation of 10^{-3} mg/l WSF, the ability to detect lower levels of WSF returned within minutes. For the levels tested, exposure to WSF did not appear to impair the ability of salmon to detect biologically relevant cues. For WSF concentrations from 10^{-7} to 10^{-3} mg/l, short-term exposure to WSF did not result in decreased chemosensory responses to amino acids. Exposures at 10^{-5} mg/1 for up to 90 min did not impair amino acid detection. The results concerning the relationship between hormone levels and chemosensory function were inconclusive.

These findings suggest that coho salmon can detect the presence of dissolved petroleum hydrocarbons at several orders of magnitude below the levels seen or predicted to cover large areas during oil spills. The salmon have the sensory ability necessary to avoid oil spills, but field studies are necessary to demonstrate whether migrating salmon will actually avoid oil-contaminated -areas. The implications of the degradation in WSF detection at higher WSF levels for avoidance of oil spills is less clear. Such degradation suggests that where migrating salmon encounter exposure levels above 10^{-3} mg/l WSF, the fish may have impaired ability to detect and avoid oil-contaminated areas.

The finding of little or no evidence for impairment of biologically relevant cues by WSF up to 10^{-3} mg/l suggests that the salmon can be expected to be able to migrate through these concentrations without becoming disoriented. Levels and durations of WSF exposure above 10^{-3} mg/l have not been tested and need investigation.

The pursuit of field studies is recommended only after more laboratory studies. The findings of Phase I shift the focus of any Phase 11 field tracking studies from investigation of potential disorientation of migrating

salmon by chemosensory disruption to investigation of avoidance. However, the possibility of disorientation through chemosensory impairment by petroleum exposure above 10^{-3} mg/liter remains open, and because of the logistical problems in applying a field treatment of sufficient magnitude to be a valid test, laboratory studies of the chemosensory effects of exposures above 10^{-3} mg/liter WSF are urged. Addressing questions of avoidance and disorientation above 10^{-3} mg/liter WSF with field tracking appears to be beyond logistical and permitting feasibility, and both questions can be addressed with laboratory studies. If laboratory studies show that the EOG response to WSF becomes increasingly impaired as WSF concentration rises, then one can reasonably expect avoidance to become increasingly unlikely as its sensory foundation is eroded. Similarly, because it is known that migrating salmon that have impaired homing cue detection become disoriented, such disorientation can be expected in the field, should laboratory studies indicate that cue detection is impaired above 10^{-3} mg/liter WSF.

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EFFECTS OF PETROLEUM CONTAMINATED WATERWAYS ON SPAWNING MIGRATION OF PACIFIC SALMON PHASE I. LABORATORY STUDIES

INTRODUCTION

Extensive offshore oil and gas development is planned for the North Aleutian Shelf and Bristol Bay, Alaska. Such development will occur in an area through which large numbers of several species of Pacific salmon migrate as they return to their home streams to spawn. Because oil exposure has been shown to cause chemosensory disruption and behavioral changes in other marine organisms (snails: Jacobson and Boylan 1973; Hyland and Miller 1979; lobsters: Atema and Stein 1974; crabs: Takahashi and Kittredge 1973; Pearson et al. 1981a; salmon: Maynard and Weber 1981; Weber et al. 1981) and because salmon depend on chemosensory detection of cues from the home stream water during their spawning migration (Johnsen 1986; Døving et al. 1985; Hiyama et al. 1966; and Bertmar and Toft 1969), there is concern that oil spilled into the path of migrating salmon may disrupt their spawning migration. The general objective of this project is to determine whether exposure to oil-contaminated water would disrupt the ability of adult Pacific salmon to migrate.

The project was designed in two phases. Phase I cons steal of laboratory studies of the effects of oil on salmon chemosensory funct on. Phase II will consist of field experiments on the effects of petroleum-contaminated waterways on salmon migration. The findings of Phase I are to be used in designing the fieldwork of Phase II.

After providing a background on the spawning migration of salmon and the potential effects of oil spills, this report presents the findings to date in the Phase I laboratory work. In raising as well as answering questions, the findings of current Phase I work indicate the need for more laboratory work as well as having implications for any fieldwork. The implications of the findings to the design of any fieldwork as well as the need for more laboratory work are discussed here in a concluding section.

BACKGROUND

Salmon Spawning Migration

The migration of salmon from the oceanic feeding grounds to the home stream spawning site involves orientation in open ocean, coastal waters, and streams. Traditionally, the oceanic and stream phases of the migration have been thought to involve different cues and mechanisms (Hasler and Scholz 1983), but chemical signals are now known to be important in both coastal waters and streams (Døving et al. 1985; Hiyama et al. 1966; and Bertmar and Toft 1969).

Sensory impairment studies, demonstrating the requirements of a functioning olfactory system for successful homing in the freshwater phase, have been conducted for many species of salmonids (Oncorhynchus kisutch, Wishy and Hasler 1954; O. nerka, Lorz and Northcote 1965; O. keta, Hiyama et al. 1966; O. tshawytscha, Groves et al. 1968; DeLacy et al. 1969; Salmo clarki, Jahn 1969; and S. trutta, Shearer 1959). In studying the stream phase of homing, Hasler and his coworkers demonstrated that coho salmon, O. kisutch, are attracted by imprinted chemical cues (Scholz et al. 1976) and that these cues are used in the upstream migration (Johnsen and Hasler 1980).

Westerberg (1982), in ultrasonic tracking studies with depth sensing transmitters, showed that the salmon's movement is closely related to the

fine-scale vertical layering of the water. These observations led to the suggestion that vertically stratified hydrographic features may be important for the salmon's orienting movements (Westerberg 1984). In ablation studies, it has been observed that anosmic salmon, i.e., fish with olfactory nerves surgically severed, do not respond to the hydrographic features as do intact fish in the same studies (Westerberg 1982; Døving et al. 1985). This observation and the experiments of Craigie (1926) and Bertmar and Toft (1969) which found that fish released in coastal waters without a sense of olfaction did not enter the home stream as well as controls, suggest that olfaction may be as important in coastal, nearshore migrations as in the stream phase of homing.

To understand how exposure to petroleum hydrocarbons might adversely effect salmon during the spawning migration, an examination of the mechanisms of chemosensory orientation is needed. Unlike other environmental signals, such as light and sound, chemical signals have no directive component and vary only in intensity. This lack of directivity therefore imposes several restrictions on possible mechanisms by which an animal might orient to a stimulus source (Johnsen 1984). Orientation to weak chemical gradients is considered unlikely (Kleerekoper 1982) so that directive information must be obtained from other cues. During the stream phase of the migration, it has been demonstrated that salmon respond to the direction of water currents. If the home stream odor is detected, the animal moves upstream with positive rheotaxis, and if the odor is absent, the fish move down current (Johnsen and Hasler 1980). Several field observations support the hypothesis that rheotaxis may be released through olfaction and thus become the main orienting cue during migration not only within a river system but in the coastal waters as well (Harden-Jones 1965; Nikoyalev 1978; Scholz et al. 1972).

For a fish to orient to a current it must have some reference system to establish its motion relative to the displacement by the water current itself. In a stream, the fish can determine the direction of the current

with respect to the bottom by tactile and/or visual signals and move upstream to the home site. However, when the fish is in open water, this reference system is lost and an alternate reference is required if the fish is to respond to water currents.

Salmon are, however, able to detect the interface between two differentially scented bodies of water. This has been confirmed through behavioral observations (Johnsen and Hasler 1980; Døving et al. 1985) and electrophysiological experiments (Døving et al. 1985). In the nearshore regions, salmon respond to horizontally stratified water masses by vertical zig-zagging at their interface. Westerberg (1984) has suggested that the salmon, after locating the interface between two adjacent water masses, make use of the local sheer currents to derive the necessary directional information needed for oriented movement.

Based on field and laboratory evidence, the following scheme for salmon homing has been proposed. When reaching the nearshore environment, salmon begin to use olfactory cues to distinguish between adjacent water masses. By monitoring sheer currents at the interface, fish can obtain directional information and move toward the home stream. After entering the home stream, the salmon continue to move against the current if the home odor is present. Through a series of simple rheotaxic behaviors related to stimulus distribution, the salmon can arrive at the home site.

This dependence on chemical signals for both the nearshore as well as the stream phases of the homing migration indicates that any change in olfactory acuity would have significant impact on the successful completion of the salmon's life cycle.

Effects of Oil on Chemoreception

Chemosensory disruption by various petroleum hydrocarbons and oil fractions has been reported in snails (Jacobson and Boylan 1973; Hyland and

Miller 1979), lobsters (Atema and Stein 1974), and crabs (Takahashi and Kittredge 1973; Pearson et al. 1981a). In early studies, the exposure regimes were not well enough defined for interpretation (National Academy of Sciences 1975) and were often greater in duration and level than that likely to be actually encountered. Under a more realistic exposure, Pearson et al. (1981a) have found chemosensory impairment in the Dungeness crab, Cancer magister. After exposing crabs in a continuously flowing exposure system to seawater contaminated with Prudhoe Bay crude oil (0.27 ppm) for 24 h and with oil still present, the proportion of crabs showing the antennular flicking response, indicative of food cue detection, was significantly reduced. Within 1 h after return to clean seawater, the chemosensory antennular response recovered. Such rapid recovery indicates that the impairment did not derive from structural damage to the chemoreceptor cells but does not indicate which of several other possibilities was the most likely mechanism. Monoaromatic hydrocarbons predominated in the oil-contaminated seawater and have been implicated elsewhere as agents of anesthesia or reversible narcosis (Crisp et al. 1967; Johnson 1977).

Only one preliminary electrophysiological study of potentia chemosensory disruption by petroleum hydrocarbons in juvenile coho salmon has been done previously. After rinsing the nares with synthetic mixture of monoaromatic hydrocarbons (4 ppm) for 20 rein, Maynard and Weber (1981) reported no significant difference in the electroencephalogram (EEG) response to the amino acid, L-serine, presented before and after exposure. There was a decreased responsiveness at the most severe exposure, but high variability and small sample size prevents adequate evaluation. Also, the exposure duration (20 rein) was rather short compared to the 12 to 24 h predicted for migrating salmon by Thorsteinson and Thorsteinson (1984) under two Bristol Bay oil spill scenarios. (For further details on potential exposures see section entitled Potential Exposures During Oil Spills.)

Several mechanisms by which petroleum hydrocarbons could disrupt chemoreception have been suggested. First, exposure to petroleum

hydrocarbons could cause structural damage to the chemosensory cells. Such damage has been inferred in the shore crab from the long period necessary for two chemosensory behavioral responses to recover after exposure to various oil fractions (Takahashi and Kittredge 1973; Kittredge et al. 1974). Second, petroleum hydrocarbons could anesthetize the chemosensory cells. Many petroleum hydrocarbons produce anesthesia in barnacle larvae (Crisp et al. 1967), and anesthesia of chemosensory cells can be inferred from the rapid recovery of chemosensory response in shore crabs exposed to single monoaromatic hydrocarbons (Takahashi and Kittredge 1973). Third, the odor of oil could mask the odor of other cues. Odor masking by oil was suggested by Atema and Stein (1974) as one possible mechanism behind a longer food finding time in American lobsters. Fourth, oil physically dispersed into the water column by turbulence could coat the chemosensory surfaces and block passage of chemical cues to the chemosensory cells. Fifth, the petroleum hydrocarbons could interact with the chemical cues to deactivate them. Such potential deactivation has been suggested by Stabell (1983) for the relatively insoluble components of oil, which theoretically could extract hydrophobic chemical cues from the water column. As far as we know, the latter mechanism of deactivation of chemical cues has received no experimental study and seems a remoter possibility for chemosensory disruption by oil than the former four mechanisms for which there is some experimental evidence.

Chemosensory disruption by oil has been observed so far only at much higher hydrocarbon concentrations than has chemosensory detection of oil. For example, impairment of food cue detection in the Dungeness crab occurred at 267 ppb of monoaromatics in a continuously flowing system (Pearson et al. 1981a) whereas detection of the water soluble fraction of a crude oil by Dungeness crab occurred at 0.4 ppb, three orders of magnitude lower (Pearson et al. 1980). The blue crab, Callinectes sapidus, detects the WSF of crude oil still lower - at 0.002 ppt (Pearson et al. 1981b). The cod, Gadus morhua, detects the WSF of diesel fuel at 0.030 to 0.300 ppb (Hellstrom and Døving 1983). The observation of saw-toothed detection curves for

hydrocarbons suggests that detection ability is acute at low concentrations but degrades at higher concentrations as a result of some toxic or anesthetic effect of the hydrocarbon (Pearson et al. 1980, 1981b).

In summary, the implications of these observations are that, at concentrations in the ppt to ppb range, marine organisms appear able to detect petroleum hydrocarbons. In the presence of hydrocarbons at higher levels, a reduced ability to detect other chemical cues can be expected, especially in the presence of oil. Anesthetic effects from monoaromatic hydrocarbons appear to have the strongest experimental support for being the mechanism behind this chemosensory disruption.

Potential Exposure During Oil Spills

Much research has been done to develop an understanding of the nature, level, and duration of hydrocarbon contamination likely to occur during and after a spill. Generally, the unique circumstances of each spill govern, in the extreme, the fate and persistence of hydrocarbons (National Academy of Sciences 1975). Once spilled, the oil is immediately subject to a variety of processes that change its physical and chemical characteristics. Spreading, evaporation, dissolution, dispersion, and sinking all act to partition various oil components among the atmosphere, sea surface, water column, and sediments (Manen and Pelto 1984). Following rapid changes in the first hours and days, this partitioning is generally complete within 10 days.

Information concerning potential exposure during oil spills comes from four sources: (1) laboratory studies of effects, (2) field studies of accidental oil spills, (3) field and mesocosm studies of experimental spills, and (4) modelling efforts. From these studies four general patterns emerge. First, the toxicity of oil derives mainly from the aromatic hydrocarbons (Anderson 1979). For chemosensory disruption, the monoaromatic hydrocarbons also seem to be an important agent (Pearson et al. 1981a) (See section entitled Effects of Oil on Chemoreception). Second, whereas the aromatic

hydrocarbons are the most toxic oil component, they are also the most volatile and soluble so that the competing processes of dissolution and evaporation determine their concentration in the water column. Volatile aromatic hydrocarbons are judged unlikely to attain high or long sustained concentrations in the water column because of rapid loss to atmosphere by evaporation (McAuliffe 1977a and b; Manen and Pelto 1984). Third, turbulence can physically disperse oil into the water column to produce higher hydrocarbon concentrations to greater depth than can be attained otherwise. Fourth, where spilled oil reaches shallow coastal waters, concentrations in sediments can become quite high and persistent (Sharp and Appan 1982). Whereas medium and coarse sediments in high energy environments can cleanse rapidly, fine-grained sediments in low energy environments appear to be "sinks" for hydrocarbon contamination (Vandermuelen 1982).

The four sources of information mentioned above also indicate potential levels and durations of exposure. In accidental spills, the concentrations of hydrocarbons in the water column have varied with the circumstances of the spill. During the last 3 days of a 21-day platform spill, McAuliffe et al. (1975) found concentrations of dissolved hydrocarbons ranging from 0.002 ppm to 0.20 ppm and estimated that half of the hydrocarbons were monoaromatics. After a North Sea platform spill, Grahl-Nielsen (1978) found hydrocarbon concentrations in the water up to 0.4 ppm. In spills where turbulence disperses oil into the water column, hydrocarbon concentrations can be higher than 0.2 to 0.4 ppm. During the AMOCO CADIZ spill, Calder and Boehm (1981) found hydrocarbon concentrations over 1.0 ppm in the water entering the Aber Wrac'h estuary and exceeding 0.5 ppm through the water column in the rest of the estuary. The presence of alkanes in subsurface water samples confirmed that the oil in the water column was present as droplets. Following the AMOCO CADIZ spill, oil-in-water concentrations of 2 to 200 ppb were observed in the nearshore zone and 30 to 500 ppb in the estuaries (Grundlach et al. 1983).

In a 10.5-barrel experimental spill, McAuliffe (1977b) found aromatic hydrocarbons to range from 0.002 to 0.050 ppm at 1.5 m after 20 min but detected none after 1 h. In experimental spills of Prudhoe Bay crude oil in mesocosms, Payne et al. (1983) found that the concentration of aromatic hydrocarbons peaked at 0.380 ppm after 12 h and then fell exponentially. For the monoaromatic hydrocarbons, substantial loss by evaporation had occurred after 1 day. For winter experimental runs, the peak concentration (460 ppm) was higher than for summer (360 ppm) (Payne et al. 1984). In a series of experimental spills treated with a chemical dispersant, McAuliffe et al. (1980) found maximum hydrocarbon concentrations in the water column of 3 and 18 ppm depending on the crude oil. After chemical dispersion, evaporation of low molecular weight hydrocarbons proceeded rapidly.

Potential spills of Prudhoe Bay crude oil have been modelled under various scenarios for Bristol Bay, Alaska (Manen and Pelto 1984; Laevastu et al. 1985). These efforts indicate two potential courses of events: either within 100 h most of the volatile oil components will have evaporated or within 48 h the spilled oil will have formed a stable emulsion ("mousse") that retards further partitioning. For 12 h after the spill and a persistent wind of 5 m/s (9.7 knots), hydrocarbon concentrations above 0.01 ppm were estimated to extend 100 m beyond the edges of a 200-m-wide slick and to a depth of 15 m. The maximum concentration was conservatively estimated to be not greater than 0.650 ppm. Modelling of a blowout scenario for the Bering Sea predicted maximum concentrations under an oil slick of 340 ppb (Laevastu et al. 1985). The modelling effort by Laevastu et al. (1985) predicted that the areas covered by oil concentrations above 1 ppb reach maximums of almost 250 km2 for a tanker accident and 500 km2 for a blowout. Concentrations above 1 ppb are predicted to continue for about 35 days for the tanker accident and slightly less than 30 days for the blowout.

The sum of these observations and estimates seems to be that maximum hydrocarbon concentrations in the water column can generally be expected to range between 0.2 and 0.65 ppm but can exceed 1.0 ppm where turbulence

physically disperses oil into the water column. For spills treated with chemical dispersants, the maximum concentrations appear to be on the order of 20 ppm. Also, exposures of more than a few days to such water column concentrations, especially for fresh oil, do not seem likely.

Know" edge of salmon spawning migration further limits the potential exposure. First, at least in the Bering Sea, homing salmon appear to be migrating in the top 5 m, the area of highest petroleum hydrocarbons concentrations (Thorsteinson and Thorsteinson 1984). Second, based on their rate of migration and assuming the salmon do not avoid oil--contaminated water, Thorsteinson and Thorsteinson (1984) estimated for two oil spill scenarios within Bristol Bay that salmon on their spawning migrations would be exposed to the widest area of oil contamination for 12 to 24 h. Third, the summer spill scenarios indicate a movement of the slick to the north side of Bristol Bay over a period of 30 days. Because salmon also concentrate on the north side of Bristol Bay (Straty 1981; Thorsteinson and Thorsteinson 1984), exposure of migrating adults is more likely to be to weathered than fresh oil. This higher probability of encountering weathered oil is simply because the volatiles will probably evaporated in 4 days (Manen and Pelto 1984) .

Potential Effects of Oil Spills on Salmon Chemoreception and Homing

There are two major ways in which salmon spawning migrations potentially can be disrupted by oil contamination: (1) through loss or degradation of the ability to detect chemical cues used in migration and (2) through avoidance of oil-contaminated areas that lay in the migratory path. These effects could reduce successful return to the home stream.

Chemosensory Disruption

Because salmon depend on the detection of chemical cues in their spawning migrations, loss of the ability to detect those cues could cause

delays in migration. The first report of disruption of a salmon spawning migration was that of Saunders and Sprague (1967) for Atlantic salmon entering a stream contaminated with heavy metals from mining operations. Whereas they explained the downstream movement of salmon as avoidance of high levels of zinc and copper in the river, a reexamination of their observations in light of present knowledge concerning salmon migratory behavior reveals a more likely and simpler explanation than avoidance. As indicated in the behavioral control model shown in Figure 1 (Johnsen 1982), salmon, upon detection of home stream odor, swim upstream against the current. In the absence of detection of home stream odor, salmon move downstream. Simple loss of the ability to detect home stream odor in the presence of the metal contamination would lead to the downstream movement of the salmon observed by Saunders and Sprague (1967). Copper and other heavy metals are known to reduce olfactory response in salmonids (Hara et al. 1976) so that loss of detection ability at the metal levels observed by Saunders and Sprague (1967) was likely.

We postulate that if oil-contaminated waterways are going to disrupt salmon migration, one such disruption will occur though an impairment of the ability to detect chemical cues with a consequent loss of ability to orient properly to current and other hydrographic features. Exposure to petroleum hydrocarbons, especially monoaromatics, has been seen to impair the ability of other marine organisms to detect chemical cues. The ability of hydrocarbons to impair chemosensory detection by salmon has not yet been adequately examined and was one of the first objects of study in this work. We hypothesize that an exposed salmon that had lost its chemosensory detection abilities would act like a surgically anosmic salmon. Such salmon do not show orientation to hydrographic features and fail to return to the home stream (Westerberg 1982; Døving et al. 1985). A salmon with impaired detection ability will show more variability in its responses to hydrographic features and, if still able to home, will do so at a slower rate because the fish is spending time swimming in search of alternate cues. If any loss or impairment of chemosensory function is observed in the laboratory, then

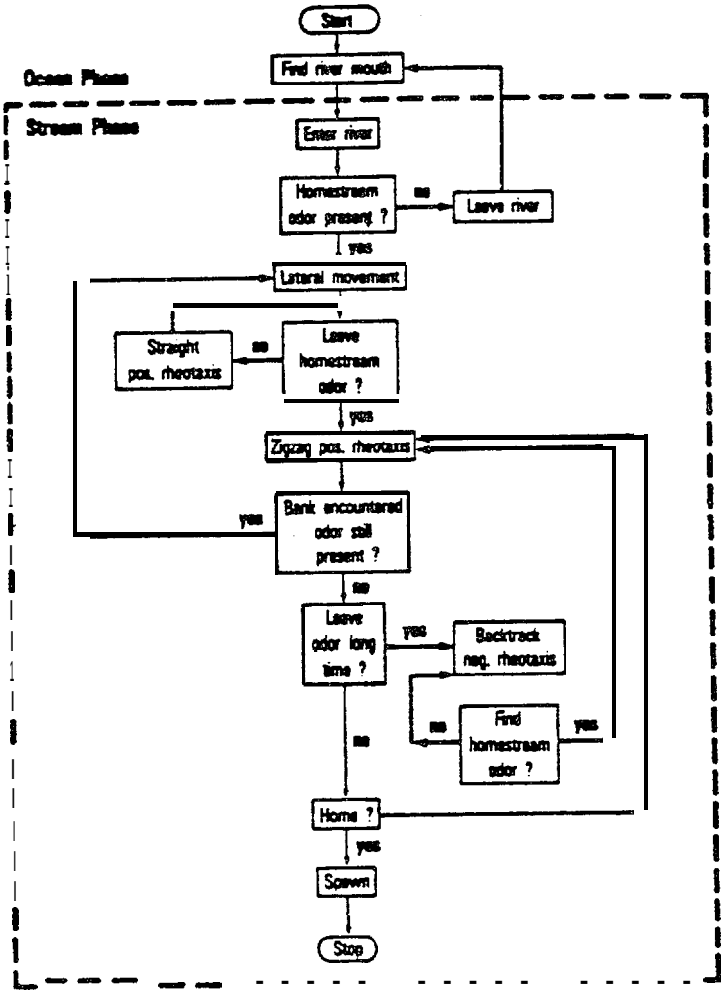

FIGURE 1. Behavioral Control Model for Upstream Movement and Homestream Selection in Migrating Salmonids (From Johnsen 1982)

disruption of orientation in the field is likely. Only if any observed impairment persists after exposure, are field tracking studies of laboratory-exposed salmon likely to observe any consequences for migratory behavior.

Indeed, in three such studies, the homing pond study and the Tulalip Creek study of Malins et al. (1985) and the Big Beef Creek study of Nakatani et al. (1985), no clear and substantial effects were observed in migrating salmon that were captured, exposed to petroleum hydrocarbons in the laboratory, marked, and then, subsequently released to continue their migration to their hatchery. In the homing pond study, the marked fish were released within the freshwater portion of their run. In the Tulalip Creek study, the marked fish were released at marine sites 1.6 and 4.7 km from the mouth of their home stream. Differences in the number of fish returning and the time course of their return were used to assess the effects of petroleum exposure on homing ability. In both studies of Malins et al. (1985), the exposure to hydrocarbons did not significantly reduce the number of returning fish. In both studies, there was time of unspecified length for recovery. In the homing pond study, fish showed no delay in return after exposure to the freshwater soluble fraction of oil up to 40 ppm for 14-18 h or after exposure to an aromatic hydrocarbon mixture up to 2 ppm for 8-22 h.

In the Big Beef Creek study of Nakatani et al. (1985), coho salmon were exposed for 1 h to an oil slick (1.6 ppm measured by IR spectrophotometry), dispersed oil (59 ppm), or dispersant alone and then released in salt water 7 km from the home stream. Of 314 fish released, 62 fish or 19.7% returned successfully with no significant difference observed among the treatment groups. Similarly, speed of return did not differ significantly among the treatment groups.

In contrast, fish in the Tulalip Creek study showed a significant delay in return (a mean of 3 days) after exposure to an aromatic mixture of 1 ppm for 8-22 h and 2 ppm for 8 h. In the Tulalip Creek study, the fish

transported the farthest distance away showed the least delay when comparing exposed fish to controls. The lesser delay could have been due to increased variability in the time for control fish to return or due to increased time for recovery from exposure effects. The actual time to return and its variance was not reported, only the difference between control and exposed fish.

The above three studies suffer from the fact that the fish did not have to pass through an oil-contaminated waterway to reach home. The experimental designs, therefore, do not directly address the question of what the behavior of salmon would be when oil contamination is present between the fish and their home. However, we do note the delayed return following exposure to aromatic hydrocarbons, an observation consistent with the notion that aromatic hydrocarbons impair chemosensory function. Because such chemosensory impairment appears to be transient, it would occur during exposure, if it does, and would be more likely to produce delay in homing rather than its failure. Because behavioral avoidance of noxious odor depends upon detection of the odor, the delay in return seen after exposure to the aromatic hydrocarbons coupled with the demonstrated anesthetic effects of the aromatics provides some evidence that behavioral avoidance of oil spills, at least when the concentrations of aromatics are high, will also be impaired because the underlying chemosensory detection is impaired.

Avoidance

Avoidance of petroleum hydrocarbons has been reported for juvenile and adult coho salmon (Maynard and Weber 1981; Weber et al. 1981). In these studies, the concentrations of a mixture of monoaromatic hydrocarbons avoided ranged from 2 to 4 ppm for juvenile salmon and 3.2 ppm for adult salmon. These concentrations are an order of magnitude higher than the maximum concentrations expected to occur under a oil slick. These high avoidance thresholds would appear to lessen the likelihood that avoidance would be a

mechanism behind delays in salmon migration, however, closer examination of the Chambers Creek study (Weber et al. 1981; Malins et al. 1985) suggests that the observations are compatible with other mechanisms and that whatever the mechanism it may have been acting at lower hydrocarbon levels than the authors' suggest.

In the Chambers Creek study (Weber et al. 1981; Malins et al. 1985), the dam had a fish ladder on each side of the creek. A mixture of monoaromatic hydrocarbons was released into one ladder, and the number of salmon ascending the treated and untreated ladder were counted. Comparisons of the numbers of fish ascending the two ladders were used to estimate that the threshold for "avoidance" of aromatic mixture was 3.2 ppm. The first problem with the study is that the experimental treatment was not confined to the treated ladder. The authors admit that all fish approaching the dam during the test periods could well have encountered the aromatic mixture downstream.

We have reanalyzed the data of the Chambers Creek experiment appearing in Table 46 of Malins et al. (1985) and have found evidence that the total numbers of fish returning to both ladders were reduced when releases of monoaromatic hydrocarbons were high (Figure 2). Regression analysis shows that the total number of returning fish has a significant but negative relation to the calculated hydrocarbon concentration ($F = 8.39$; d.f = 1,10; $p < 0.05$; R-squared = 45.6%). The regression of the total number of returning fish against the measured hydrocarbon concentration is also significant but with more variance (R-squared = 37.9%). In Figure 2, one outlier is evident, and this data point derives from the first day of testing. All others come from a time after hydrocarbons had been introduced into the stream. Multiple regression analysis with time before or after first introduction of the hydrocarbons as a variable again gives a significant regression between total number of returning fish and the two variables, calculated hydrocarbon concentration and time before or after introduction ($F = 18.04$; d.f. = 2,9; $p < 0.01$; R-squared = 80.0%). This reanalysis provides strong circumstantial evidence that the aromatic hydrocarbons released into Chambers Creek were

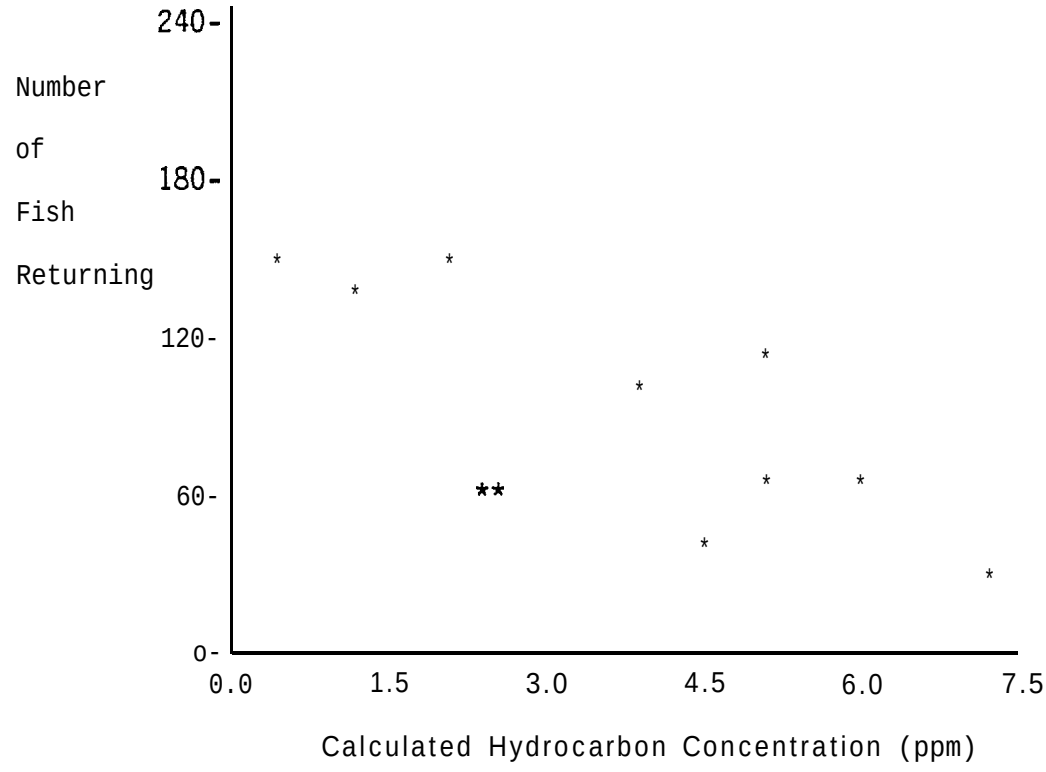

FIGURE 2. The Total Number of Salmon Returning to Both Ladders Versus the Calculated Hydrocarbon Concentration in the Chambers Creek Experiment (Malins et al. 1985)

reducing the overall return of the salmon if information was available on the total days when no hydrocarbons were released. The case would be even stronger number of fish returning on the Unfortunately, the authors only report the overall return under the no-release conditions rather than the daily returns.

Whereas the authors claim the Chambers Creek results to show that fish avoid a mixture of aromatic hydrocarbons at 3.2 ppm and above, our reanalysis suggests something different. The total number of fish returning to the creek showed a significant negative relationship with the released amount of the mixture of aromatic hydrocarbons. Such observations could be due to behavioral avoidance of the hydrocarbon mixture downstream rather than at the dam or due to impairment of the ability to sense the home stream water. As our well-established behavioral control model (Johnsen 1982; Figure 1 here) indicates, loss of the ability to detect home stream water will lead to downstream movement of salmon, an event that can be misconstrued as "avoidance." Studies with other species demonstrate that monoaromatic hydrocarbons can impair chemosensory function.

In light of this, we suggest an alternative explanation of the Chambers Creek results. On the first day of testing, no aromatic hydrocarbons had been released into the stream so that the normal number of returning fish were at the dam. Upon release of the monoaromatics, those fish on the treated side of the dam where the hydrocarbons were released moved downstream because they lost the ability to detect the home stream odor Those fish already at the dam on the untreated side continued to detect the stream odor and moved upstream to be counted. Those fish moving upstream well below the dam were encountering some concentration of the aromatic hydrocarbons. Fish encountering a level sufficient to impair detection of home stream odor moved downstream. On the days following the first day of release, the number of fish coming to the dam was reduced to the extent that stream water below the dam contained an aromatic hydrocarbon level sufficient to impair home odor detection. Because the hydrocarbons were probably not evenly distributed

across the stream downstream, some fish were continuing to move upstream to the dam. At the dam, the scenario for the first day was again repeated. The implication of this alternative explanation is that the aromatic hydrocarbons were not acting as noxious odor to which the salmon were exhibiting avoidance behavior but rather were acting as disrupters of the chemosensory detection of home stream odor that, in turn, led to downstream movement of the fish. The point to be remembered from this reanalysis is that an observed change in orientation behavior could have been caused by one of two equally plausible mechanisms: avoidance or chemosensory impairment. Whatever the mechanism, a closer examination of the Chambers Creek results (Weber et al. 1981; Malins et al. 1985) reveals that the released hydrocarbons were apparently active at levels below that indicated by the authors' original interpretation.

Potential Effects of a Delay in Spawning Migration

The concern is that a delay in the spawning migration might adversely affect reproductive success. It is difficult to determine to what extent delays in the spawning migration might influence reproductive success. This difficulty stems largely from the anticipated lag time between the encounter with an environmental disturbance and the actual spawning period. Recent experience in the Mount St. Helens region has demonstrated that salmon are quite flexible in their spawning behavior. When finding their homestream tributary blocked or obliterated by volcanic ash, fish moved to alternate sites and successfully spawned later than normal (Whitman et al. 1982).

In contrast to delays in freshwater, significant delays at sea or in the estuarine regions might have some effects on reproductive success. It appears that the physiological changes that occur upon salmon's entrance into fresh water may also influence the final sexual maturation (Sower and Schreck 1982). When the salmon normally enters freshwater from the ocean, they must undergo osmoregulatory changes; these changes affect, or are affected by, the endocrine system (Woodhead 1975). Thus, if the returning salmon are denied normal entry into freshwater, the endocrine system may not be able to respond

properly. In an experimental study in which fish were retained in seawater during the spawning season, Sower and Schreck (1982) observed modified hormone profiles, dehydrated eggs, small amounts of seminal fluid, incomplete ovulation, low egg survival, and high adult mortality. Such findings suggest that osmoregulatory factors strongly influence the maturational process of salmon and that delays confining migratory salmon to saltwater environments may result in hindered reproductive development.

Although the available information is suggestive, predictions of possible effects on reproductive success cannot be made until the length of the migratory delay is determined. In particular, the hormonal and maturational status of the fish at the time of delay would be an important determinant of reproductive success. Length of delay can be estimated for given exposure levels and durations should chemosensory disruption prove to be the mechanism underlying delay or estimated from time spent in avoidance behavior should avoidance behavior be evident at particular levels of oil contamination. To assess potential effects on reproductive success, effects thresholds must then be related to oil spill scenarios that indicate the level, extent, and duration of oil-contamination of migratory pathways. Comparison of the levels and durations of oil contamination likely to be encountered with the levels and durations producing delays would indicate the expected length of delay. The oil spill scenarios would need to be examined to indicate the likelihood that the delay would occur in saltwater and be of sufficient duration to produce the effects described by Sower and Schreck (1982).

SCENARIOS OF IMPACT FROM OIL SPILLS

What is important to realize is that the studies discussed here rest on the behavioral model developed over many years for the mechanisms in salmonid migration (Johnsen and Hasler 1980; Johnsen 1984; Johnsen 1986) and briefly described above. In the freshwater and nearshore phases of migration, salmon depend on the chemosensory detection of chemical cues to orient their

movements toward and up the home stream (Døving et al. 1985; Hiyama et al. 1966; Bertmar and Toft 1969; Westerberg 1982, 1984; Harden-Jones 1965; Nikoyalev 1978; Scholz et al. 1972). Petroleum has an odor, and, under some circumstances, can disrupt the chemosensory detection of other odors (Hell strom and Døving 1983; Pearson et al. 1980, 1981a, b). These facts allow the development of several plausible scenarios for the effects of contamination of waterways by petroleum on salmonid spawning migration. The project was intended to provide data to indicate which of the following scenarios are the most likely:

- Detection and avoidance of petroleum

  In this scenario, migrating salmon detect the presence of petroleum and avoid the contaminated area. Consequently, the probability of exposure to petroleum hydrocarbons is decreased, but migration might be delayed. Any delay in migration would be a function of extent of the contaminated area and the time necessary to move around it. The laboratory studies of Phase I addressed the threshold for detection of petroleum hydrocarbons.

- Detection of petroleum hydrocarbons but no avoidance

  Migrating salmon may detect the petroleum contamination but not avoid the contaminated area. If the fish swim through the area, delays in migration would presumably not occur. However, exposure would occur and could increase the probability of acquiring a flavor taint depending on the levels and time duration of exposure. The fieldwork of Phase II is intended to address questions concerning avoidance.

- No detection of petroleum hydrocarbons and, therefore, no avoidance

  Migrating salmon may not detect petroleum hydrocarbons under two conditions: first, when the petroleum hydrocarbons concentrations are

below the detection threshold (the low threshold found in this study will be discussed later) and, second, when the petroleum hydrocarbons concentrations are high enough to disrupt their detection. Should no detection occur, avoidance of the contaminated area appears unlikely. The detection thresholds are quite low so that lack of avoidance of areas with petroleum hydrocarbons concentrations at and below the threshold cause no concern. Lack of avoidance of petroleum hydrocarbons concentrations at which detection is degraded depends on the steepness of the gradient encountered as the fish approaches the contaminated area. It is conceivable that a fish encountering a steep gradient may have only a brief time to detect the petroleum hydrocarbons before its detection ability is depressed. Should the fish not avoid a contaminated area, the questions arising concern the effects of exposure, the extent of which would depend on the exposure level and duration. The laboratory studies of Phase I addressed the question of degradation of chemosensory detection at higher levels of petroleum hydrocarbons.

- Disorientation caused by disruption of the detection of homing cues from exposure to petroleum hydrocarbons

  Because the detection of homing cues is necessary for the appropriate orientation of salmon during migration, degradation of homing cue detection during exposure to petroleum hydrocarbons can be expected to lead to disorientation in the migrating salmon. Should petroleum hydrocarbons impair homing cue detection, delays in migration would be expected, and the degree of delay would depend on the extent of the spill and the duration of disorientation. The laboratory studies of Phase I addressed in part questions concerning the ability of salmon to detect biologically relevant cues under exposure to petroleum hydrocarbons.

OBJECTIVES

The specific research questions addressed in Phase I were the following:

1. Can salmon detect oil?
   a. If so, at what concentration?
   b. If so, is there a degradation of chemosensory detection at high concentrations due to anesthetic or toxic action?
2. Does oil exposure cause loss or impairment of chemosensory detection of other chemical cues?
   a. If so, at what level and duration of exposure?
   b. If so, does the effect occur only in the presence of oil?
   c. If the effect continues after return to clean water, how long does recovery take?

Also, a complementary objective was to determine how "spawning pressure" or motivational state might influence salmon responses to petroleum hydrocarbons. The question of how motivational state is related to spawning condition and migratory behavior can not be directly addressed because there are no techniques for measuring motivational state or any adequate physiological assays of early spawning condition. Our approach to the question of how motivation might be influencing the various responses of salmon was to attempt post hoc correlations of olfactory responses observed on a particular date with the hormonal status of that fish measured using radioimmunoassay (RIA) techniques. Another objective of the laboratory work of Phase I was to provide information to design the field tracking studies of Phase II.

Here we report on findings from four areas:

1. The chemosensory detection threshold for the water-soluble fraction (WSF) of Alaska North Slope (ANS) crude oil in adult coho salmon

2. Changes in chemosensory function of adult coho salmon at higher concentrations of the WSF of ANS crude oil

3. Effects of short-term exposure of adult coho salmon to the WSF of ANS crude oil on detection of an amino acid mixture

4. Detection of an amino acid mixture by adult coho salmon during exposure to the WSF of ANS crude oil.

Findings in all four areas derive from use of the same basic EOG technique with different protocols of stimulation and experimental treatment of the fish. Also, we report inconclusive results concerning the relationship between hormone levels and chemosensory function.

MATERIALS AND METHODS

CAPTURE AND HOLDING

Adult male coho salmon, *Oncorhynchus kisutch*, were captured by hook and line from salt water in the Strait of Juan de Fuca and by seine from the holding basin of the Dungeness Fish Hatchery operated by Washington Department of Fisheries. Some laboratory-reared 2-year-old chinook salmon, *O. tshawytscha*, were used in the preliminary testing done to modify the EOG apparatus for use with the WSF of crude oil but are not included in the data analysis. The fish were held in 1800-gal circular tanks supplied with aerated well water flowing at a rate of 15 l/rein. Coho salmon were not fed during captivity. For transferring fish at various stages in the project, a specially designed sling was used. Unfortunately, the fish captured in salt water were lost through a failure of the water supply to the holding system just at the time when they became no longer available in salt water. Fish weights, capture dates and holding time for the fish used in the experiments are given in Table 1.

PREPARATION OF FISH FOR EOG EXPERIMENTS

For the electrophysiological experiments, the fish were anesthetized with MS-222 (tricainemethane sulphonate, 1:20,000 w/v dilution), immobilized with Flaxedil (gallamine triethiodide, 0.1 mg/100 g body weight) and fastened in a Plexiglas fish holder (Figure 3). Wet sponges, held in position with Velcro straps, were used to keep the fish restrained in the proper position. Aerated well water containing MS-222 was perfused through the mouth over the gills *via* a recirculating system. Perfusion water was maintained below 5°C with an ice bath. At the end of each day's experimentation, a cardiac

TABLE 1. Biographies of Individual Fish Used in the EOG Experiments. The column entitled RIA provides the results of the radioimmunoassay on circulation hormones estradiol (E2) and testosterone (T). The column entitled IR gives the concentration of total hydrocarbons measured by infrared (IR) spectrophotometry for each WSF batch.

Fish # Species	Capture Date	Test Date	Wt. (g)	Length (cm)	WSF batch	IR mg/l	RIA E2 pg/ml	RIA T ng/ml	Tests performed	Quality Control Acceptable (Yes/No)
86-1 Chinook	Lab. reared	10-27-66			16	8.9			Threshold WSF 3,2,1 dil.	Prelim. test fish
86-2 Chinook	Lab. reared	10-26-86			16				Threshold WSF 3,2,1 dil., short-term effects	Prelim. test fish
86-3 Coho	9-23-66	10-29-86			17	6.1			Fish died	-----
66-4 Chinook	Lab. reared	10-30-86			17				Threshold WSF 12,9,6 dil.	Prelim. test fish
66-6 Chinook	Lab. reared	10-31-66			16	4.7			Threshold WSF 9,6,3 dil., short-tern effects	Prelim. test fish
86-6 Coho	10-31-66	11-1-86	1590	55	18				Fish died	-----
66-7 Coho	10-31-66	11-1-86	1615	60	18				Fish died	-----
86-6 Coho	10-31-86	11-4-66	1472	64	19	4.8			Fish died	-----
86-9 Coho	10-31-66	11-4-86	1557	64	19				Threshold WSF 9,6,3,1 dil.	Yes?
86-10 Coho	10-31-66	11-6-86			19				Threshold WSF 12,9,6,3 dil., short-tern effects	No-System contain.
66-11 Coho	10-31-66	11-7-66	1730	68	19				Threshold WSF 12,9,6,3,2,1 dil.	No-System contain.
66-12 Chinook	Lab. reared	11-12-66			20	4.5			Fish died	-----
66-13 Chinook	Lab. reared	11-12-66	132	22	20				Threshold WSF 9,6,3 dil., short-term effects	Prelim. test fish
66-14 Chinook	Lab. reared	11-13-66			20				Fish died	-----
86-15 Chinook	Lab. reared	11-13-86	109	23	20				Threshold WSF 9,6,3,2,1 dil., short-term effects	Prelim. test fish
66-16 Coho	10-31-86	11-14-66	1410	63	20		{.1	26.76	Threshold WSF 9,6,3,2,1 dil., short-term effects	Yes
66-17 Coho	10-31-86	11-18-86	1355	64	21	5.5	0.61	28.12	Threshold WSF 7,6,5,4,3 dil., short-tern effects	Yes
66-18 Coho	10-31-66	11-19-86	1190	62	21				Threshold WSF 12,10,6,7 dil.	No-System contam.
66-19 Coho	10-31-86	11-20-86	1780	60	21		(.1	29.28	Threshold WSF 7,6,5,4,3 dil., short-term effects	Yes
66-20 Coho	11-16-66	11-21-86	1628	68	21		(.1	21.08	Threshold WSF 7,6,6,4,3 dil., short-term effects	Yes
66-21A Coho	11-16-66	12-2-86	2505	64	22	5.4	(.1	30.24	Threshold WSF 7,6,5,4,3 dil., short-tern effects	Yes
86-21B Coho	11-18-66	12-3-86	2060	80	22				Fish died	-----
66-22 Coho	11-18-86	12-4-86	1600	66	22		(.1	33.36	Threshold WSF 7,6, S,4,3 dil.	No-System contain.
66-23 Coho	11-16-86	12-5-66	1485	56	22		(.1	44.36	Threshold WSF 7,6, S,4,3 dil., short-tern effects	No-System contain.
86-24 Coho	11-16-86	12-9-66	1455	56	23	3.9	(.1	28.60	Threshold WSF 7,6,5,4,3 dil., short-tern effects	Yes

TABLE 1. Continued

Fish #	Species	Capture Date	Test Date	Wt. (g)	Length (cm)	batch	WSF mg/l	RIA IR E2 pg/ml	RIA T ng/ml	Tests performed	Quality Control Acceptable (Yes/No)
86-26	Coho	12-3-86	12-10-86	3200	72	23		(.1	61.32	Exposure effects	Yes
86-26	Coho	12-3-86	12-11-88	2359	64	23		<.1	44.52	Exposure effects	Yes
86-27	Coho	12-3-86	12-12-86	1566	67	23		(.1	47.92	Exposure effects	Yes
86-26	Coho	12-3-86	12-13-86	1555	57	23		(.1	49. S2	Exposure effects	Yes
86-29	Coho	12-3-86	12-14-86	1540	66	23		(.1	25.04	Exposure effects	Yes
86-30	Coho	12-3-88	12-15-86	1766	60	23		(.1	23.81	Exposure effects	No-System contain.
86-31	Coho	12-3-86	12-17-86	1455	63	23		(.1	9.84	Exposure effects	Yes

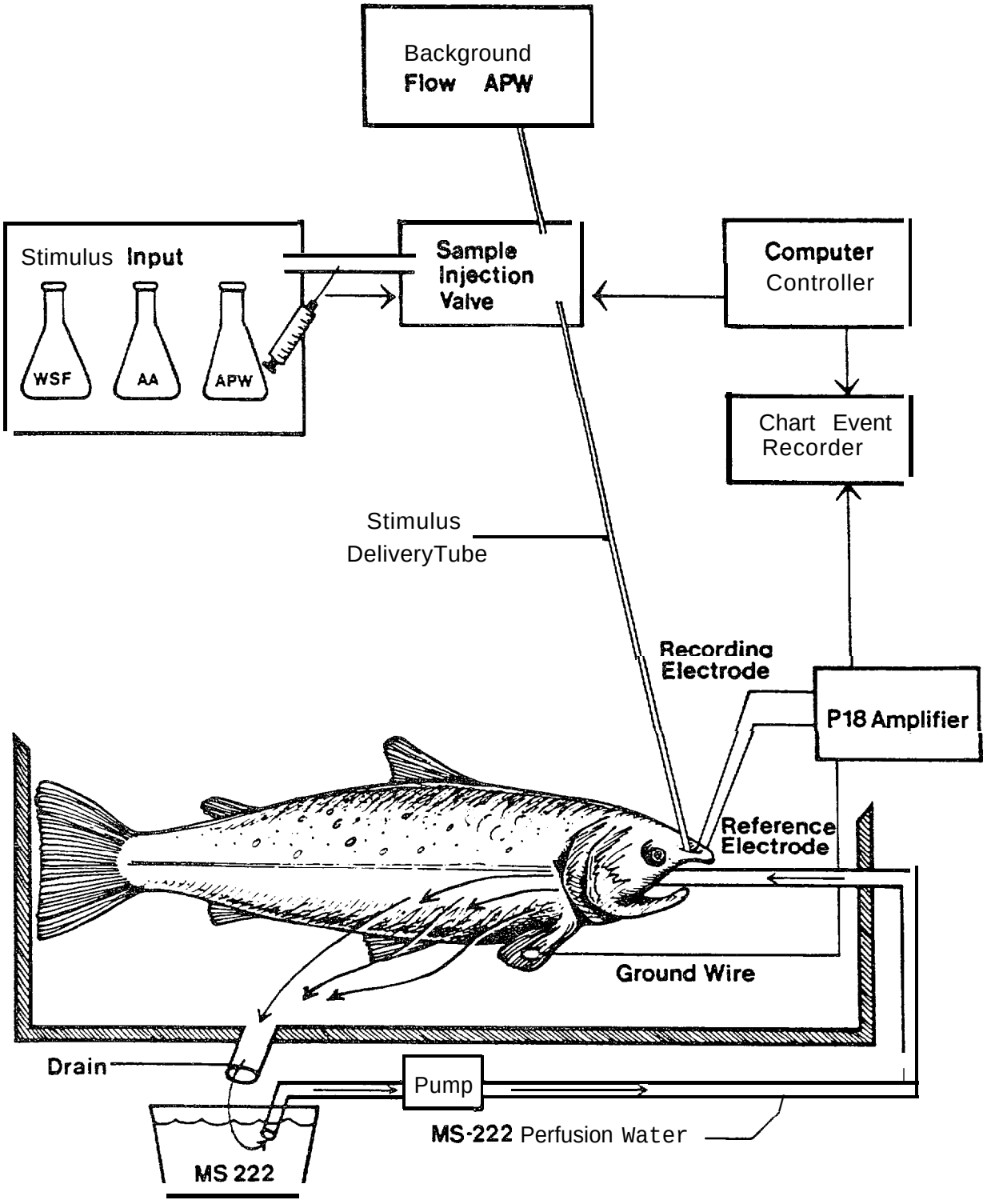

FIGURE 3. The Apparatus for the Electro-Olfactogram (EOG) Experiments with Pacific Salmon

puncture was performed to obtain blood for later RIA analysis for the hormones estradiol and testosterone.

EOG RECORDING TECHNIQUE

To record the EOG response from an individual fish prepared as described above (Figure 3), the olfactory rosette was exposed by removing with ophthalmic scissors the flap of skin forming the incurrent and excurrent nasal pores. The underwater EOG (electro-olfactogram) is a slow potential change in the olfactory mucosa elicited by chemical stimulation (Silver et al. 1976) and was recorded by placing a Ringer-agar filled glass capillary, bridged to a calomel electrode, in the water flowing over the olfactory mucosa. All recordings were made from the center of the rosette along its midline. The reference electrode of similar construction was positioned on the head adjacent to the olfactory capsule. The fish was grounded byan alligator clip to the pectoral fin. The electrodes were dc coupled to a Grass P-18 preamplifier and the signal was displayed on an oscilloscope and Western Graphtec WR7500 pen recorder.

EXPERIMENTAL SOLUTIONS

To maintain control of the ionic and organic constituency of the water flowing over the olfactory mucosa, Artificial Pond Water (APW) (0.3 mM NaCl; 0.02 mM KC1; 0.2 mM $CaCl_2$; 0.2 mM $NaHCO_3$ in distilled deionized water) was used for background flow and to make up all stimuli. The use of freshwater as the background flow does not diminish the applicability of these results to the marine and estuarine situation. First, the olfactory receptor cells are covered with a coating of mucus so that the olfactory cilia and presumptive receptor sites are chemically isolated from the surrounding media (Tucker 1983) . In fact, it has been demonstrated that the responses of receptor cells to odor substances are not significantly modified after changes in ion concentrations (Suzuki 1978). Labyrinth cells found in

olfactory mucosa have a chloride cell-like structures that serve as excretory cells for osmo- and ionregulation. In this way, these cells may allow the olfactory organs to function optimally in waters of different salinity (Bertmar 1982). The effect of increased ions in salt water is to shunt the EOG signal (Oshima and Gorbman 1966). Therefore, the conductivity of seawater decreases the absolute amplitude of the recorded signal. However, the signal-to-noise ratio is not degraded in going from freshwater to marine fish. The slope of the olfactory response-concentration curves of marine fishes are similar to those obtained for freshwater fish (Tucker 1983). Additionally, the olfactory responses of Atlantic salmon stimulated with seawater samples (Døving et al. 1985) were similar for the same species stimulated with freshwater samples (Sutterlin and Sutterlin 1971).

The WSF of fresh ANS crude oil was prepared following methods similar to Anderson et al. (1974). Three liters of APW were added to a glass carboy containing a Teflon stir bar and glass siphon tube. An aliquot of 333 ml of oil were carefully added to the surface of the water layer, and the carboy was sealed with a stopper covered with aluminum foil and through which the siphon tube extended to below the oil phase. The mixture was stirred for 20 h at a rate allowing the oil vortex to extend no more than 20% of the distance to the bottom of the bottle. After mixing, the oil and water phases were allowed to separate for 4 h. The water was siphoned from below the oil phase and filtered through a 0.45-micron filter under low pressure to remove any remaining oil droplets. Serial dilutions of WSF were made daily from a half strength stock WSF solution using fresh chilled APW. The WSF dilutions were kept in an ice water bath during use. The stock WSF was analyzed by capillary gas chromatography for diaromatic and triaromatic hydrocarbons (Bean et al. 1980). Monoaromatics were analyzed by gas chromatographic headspace analysis using methods modified from Wylie (1985). Total extractable hydrocarbons were measured from the half strength stock WSF solution using IR spectrophotometry (Bean et al. 1980). Table 2 gives the composition of the WSF of ANS crude oil. The monoaromatic hydrocarbons constituted 97% of those

TABLE 2. The Composition of Stock Solutions of WSF of Alaska North Slope Crude Oil. Sample size was 8 for monoaromatics, 3 for polynuclear aromatics, and 8 for IR spectrophotometry analysis.

	mean ug/l	S. D.
Monoaromatics		
benzene	7.1s8	0.641
toluene	5.163	0.137
ethyl benzene	0.361	0.009
m+p xylene	1.120	0.028
o-xylene	0.529	0.013
1,2,4-trimethyl benzene	0.154	0.004
1,2,3-trimethyl benzene	0.098	0.004
Total	14.588	0.836
Polynuclear aromatics		
naphthalene	0.1938	0.0474
2-methyl naphthalene	0.1011	0.0225
1-methyl naphthalene	0.0657	0.0143
2-8 & 2-7 dimethyl naphthalene	0.0170	0.0042
1-6 dimethyl naphthalene	0.0137	0.0027
1-4 dimethyl naphthalene	0.0081	0.0017
1-6 dimethyl naphthalene	0.0077	0.0019
1-2 dimethylnaphthalene	0.0073	0.0018
1-7 & 1-8 dimethyl naphthalene	0.0011	0.0015
2,3, 5-dimethylnaphthalene	0.0056	0.0015
phenanthrene	0.0039	0.0004
2-methyl phenanthrene	0.0000	0.0000
1-methyl phenanthrene	0.0004	0.0002
3,6-dimethyl phenanthrene	0.0000	0.0000
Total	0.4253	0.0956
TOTAL HYDROCARBONS	15.0131	0.9312
IR analysis (Total extractable hydrocarbons)	5.9	0.91

measured by GC. Saturate hydrocarbons were not analyzed because their concentrations would have been less than detectable. The stock solutions averaged 5.9 mg/1 as measured by IR and 15.0 mg/l as measured by glass capillary GC and GC headspace analysis.

The amino acid stimulus was composed of a mixture of L-serine, L-cysteine, and L-alanine. Stock solutions of 10^{-2} Mwere made weekly in APW. Test solutions were diluted daily with fresh APW to a 10^{-4} M concentration.

METHOD OF STIMULATION

One ml of each stimulus sample was delivered via a sample injection valve (Rainin Instrument Co., Inc. Model 201-14) to the olfactory capsule. A mariott bottle was used to maintain a constant background flow (6 ml/min) of APW over the olfactory mucosa. For Fish 86-1 to 86-15 (Table 1), the sophisticated automatic injection system originally proposed was used but proved unacceptable because there was bleed-through of the WSF solutions from one presentation to the next as well as interactions between the WSF and system components. For Fish 86-12 and above, a manual injection proved to yield acceptable results. For each sample presentation producing data reported here, the test stimulus was loaded into the sample injection loop by hand using a disposable syringe. A Commodore VIC-20 microcomputer started the pen recorder, delivered the stimulus, and placed an event mark on the chart. The computer maintained the interstimulus interval of 180 sec during which time the nasal capsule was flushed with the background solution.

Using color densitometry, dilution of the stimulus by the background flow was determined. Peak concentrations experienced by the fish were 77% of the injected concentration. Therefore, all concentrations of the amino acid stimulus actually experienced by the fish would presumably be 23% lower than the concentration injected. Besides being diluted by the background flow, the injected WSF suffered additional loss, which was determined by headspace

analysis of samples taken at various points in the delivery system. Peak concentrations of WSF experienced by the fish were reduced by an additional 38±5% of the injected value. The WSF concentrations given in the subsequent figures and tables have been adjusted for loss in the delivery system by multiplying the injected value by the percentage, 47.7%.

PROTOCOLS OF STIMULATION

Different protocols of stimulation were used to address different questions concerning the effects of WSF on the salmon chemosensory function.

For determination of WSF thresholds and changes in detection at higher concentrations, WSF dilutions were presented in an ascending order to coho salmon (Fish 86-16 to 86-24 in Table 1). For Fish 86-17 and 86-19 to 86-24, the series was as follows:

blank
10^{-4} M amino acid
blank
10^{-7} mg/l WSF
blank
10^{-6} mg/1 WSF
blank
10^{-5} mg/l WSF
blank
10^{-4} mg/1 WSF
blank
10^{-3} mg/l WSF
blank
10^{-4} mg/l WSF
blank
10^{-4} M amino acid
and blank.

Three series were presented to each fish. In each series, an APW blank alternating with the WSF stimulus served as a control while an amino acid mixture served as an internal standard. This standard allowed us to normalize data across fish and through the time course of experiments. This was necessary because the amplitude of the dc EOG response is dependent on the position of the recording electrode relative to the olfactory epitheliums. Because decreases in the EOG response were being observed above 10^{-4} mg/l WSF, another 10^{-4} mg/1 WSF concentration was presented in the series to determine whether the EOG response to 10^{-4} mg/l WSF was degraded following presentation of 10^{-3} mg/l WSF.

To examine the effects of short-term exposure to WSF on the detection of amino acids, the response to amino acid mixtures following stimulation by WSF was measured. The series was as follows:

blank
10^{-4} M amino acid
10^{-7} mg/1 WSF
10^{-4} M amino acid
10^{-6} mg/l WSF
10^{-4} M amino acid
10^{-5} mg/1 WSF
10^{-4} M amino acid
10^{-4} mg/l WSF
10^{-4} M amino acid
10^{-3} mg/l WSF
10^{-4} M amino acid
10^{-4} mg/1 WSF
10^{-4} M amino acid
and blank.

As in the WSF threshold experiments, increasing concentrations of WSF were presented to the fish. Following the normal rinsing during the interstimulus

interval, amino acids were presented rather than a blank. The amplitude of responses to these stimulations were compared to those presented before the effects trials. A single effects series was presented to each fish at the end of three series of presentations for threshold determination.

The original plans also included using a heart rate conditioning (HRC) system to determine the effects of the presence of petroleum hydrocarbons on chemosensory detection of other biologically significant chemicals. Seal ing up the HRC system from designs successfully used with small fish to ones capable of using large adult salmon proved to require more developmental work than the limited availability of the salmon would permit. We were able to successfully condition laboratory-reared 2-year-old chinook salmon and to obtain heart records from adult coho salmon, but we were unable to subject adult coho salmon to the conditioning protocols because of the time limitation on fish availability. To address the questions concerning the effects of WSF exposure on detection of biologically relevant cues, we used the EOG technique.

Using the EOG technique, experiments were conducted to determine if the fish could detect meaningful biological stimuli in the presence of WSF. For Fish 86-25 to 86-31, EOG responses were measured when the background flow over the olfactory mucosa was first, APW, second, 10^{-5} mg/1 WSF, and, finally, APW. With APW as background, the EOG response was measured for the following series: blank, 10^{-4} M amino acid, and 10^{-5} mg/l WSF. Following these presentations, the background flow was replaced with a WSF solution equal in concentration to the previously tested solution. After 30 min and still with a background of 10^{-5} mg/l WSF, stimuli were presented in the following series: 10^{-5} mg/l WSF, 10^{-5} mg/l WSF and 10^{-4} M amino acid together, 10^{-4} M amino acid, blank, and 10^{-5} mg/l WSF. The background was then returned to APW, and, after 30 rein, stimuli were presented in the series: blank, 10^{-4} M amino acid, and 10^{-5} mg/l WSF. Three trials of each stimulus under each of the three different background flows were conducted. The mean responses during these three stimulation series were then compared.

DATA ANALYSIS

Magnitude of the EOG response was measured from baseline to the peak of the response. Responses are expressed in arbitrary units that may be converted to absolute units (mV) by comparing the size of known calibration signals to the response to chemical stimuli [1 mV = 12 divisions (arbitrary unit)]. Examples of the EOG responses in a stimulus series are shown in Figure 3. Concentration values for the amino acid mixture in Figure 3 are expressed as the concentration presented, without accounting for delivery dilution. Concentration values for the WSF presented in Tables 3 to 6 and Figures 4 to 7 have been adjusted for dilution and loss in the delivery system.

QUALITY ASSURANCE

In addition to the chemical analysis reported elsewhere, two types of tests were performed so that the responses from different fish could be compared. The amino acid solution served as an internal standard calibration reference. All responses are expressed in terms of the individual animal's response to that standard stimulus. In addition, contamination of the test apparatus and the glassware used was evaluated by measuring responses to APW that had been held in glassware to be used in the testing. The EOG responses of fish were examined, and, if abnormally high responses to blanks occurred or no change in response was observed over a wide range of concentrations, the fish was dropped from analysis. Generally, fish used after the switch to manual injection with disposable syringes (at and above Fish 86-16 for coho salmon) yielded acceptable data (Table 1).

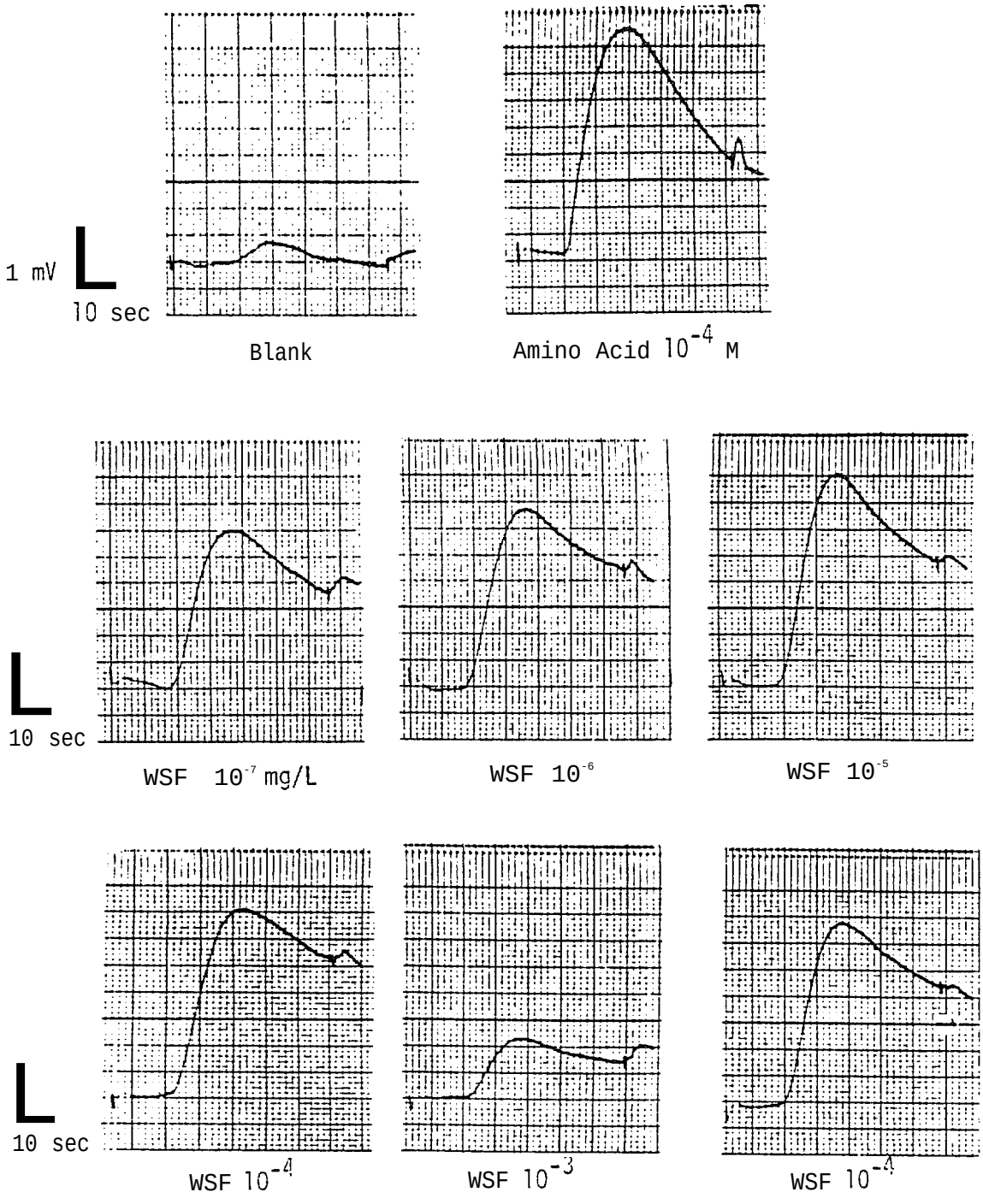

FIGURE 4. EOG Responses to a WSF Concentration Series Presented to Fish #86-20

RESULTS

THRESHOLD FOR DETECTION OF WSF

A primary task was to determine the olfactory detection threshold of WSF using the EOG method with the specific protocols described above. Threshold is defined as that concentration of a stimulus that can be perceived above the ambient noise level. In the EOG recording technique, the noise level is established by measuring the response of the animal to a blank. This blank is the same water in which the stimulus of interest is prepared. Additionally, an ideal blank is as similar to the background flow as possible so that responses to inadvertent contamination are minimized by sensory adaptation.

There exist two common methods to determine the detection threshold. The first involves presenting samples in increasing concentration steps from very low to higher in an attempt to bracket the noise level. Threshold s then determined to be the concentration that elicits a response slightly greater than the background response. Difficulties in this approach are selecting appropriate step sizes and ensuring that the response to backg ound is minimized.

A more common method involves determining the slope of the olfactory response function and calculating the concentration that produces a response equal to that of the background. The existence of a response function over a broad range of concentrations in the peripheral olfactory system is well accepted (Tucker 1983), and a law of logarithmic dependence has been confirmed in fish and for a variety of stimuli (Caprio 1983). The value of having a response function is that one can extrapolate from response points to the limit imposed by noise (control response) for a threshold

concentration determined electrophysiologically. Each stimulus has a particular slope for its linear response function. This slope is determined by measuring responses to a relatively few concentrations of the stimulus and using linear regression of logarithmically transformed responses and concentrations to calculate the equation of the line. This approach has the advantage that fewer concentrations need to be presented and one does not need to have an estimate of the threshold in order to make the appropriate dilutions to bracket the threshold.

Using the extrapolation approach, we presented a series of WSF dilutions chosen in the lower concentration range of known olfactory stimuli for salmonids. For each fish, mean responses to three replicate presentations of each concentration were measured and normalized by expressing responses as percentage of the amino acid response (Table 3). After logarithmic transformation of the variables, linear regressions were calculated for both individual fish means as well as the population mean of individual means.

It is observed from these data that calculated thresholds for individual fish and the population as a whole give extremely low values; calculated thresholds ranged from 11 to 33 log dilutions of the stock WSF solution. Obviously the lower estimates are unrealistic values. A 10^{15} dilution of WSF would have less than 10 hydrocarbon molecules of the molecular weight of naphthalene in the olfactory capsule. Examination of the plots of the concentration-response curves indicates that, unlike previously studied stimuli, the response function does not follow the law of logarithmic dependence (Figures 5 and 6). This deviation will be discussed in greater detail below in the section where the responses to the higher concentrations are considered.

Estimates of the detection threshold can be made on the basis of a number of observations. The most effective known chemical stimuli for fish and for salmonids in particular include amino acids and bile acids. Tucker (1983), in reviewing the physiology of fish chemoreception, observed

TABLE 3. EOG Responses of Coho Salmon to Three Replicate Presentations of a Series of WSF Concentrations.

EOG RESPONSE

	Presentation Series					% LOG
FISH #86-17	1	2	3	MEAN	LOG MEAN	MEAN M
BLK				2.2	0.34	28
M	16	21	14	17.0	1.23	100
-7	11	7	11	9.7	0.99	80
-6	21	25	19	21.7	1.34	109
-5	30	24	19	24.3	1.39	113
-4	29	27	28	28.0	1.45	118
-3		13	14	13.5	1.13	92
-4		22	23	22.5	1.35	110
FISH #86-19						
BLK				1.1	0.04	3
M	34	26	29	29.7	1.47	100
-7	22	24	24	23.3	1.37	93
-6	24	26	26	26.3	1.40	95
-6	28	28	26	27.3	1.44	98
-4	22	25	23	23.3	1.37	93
-3	8	8	9	8.3	0.92	83
-4	20	24	30	24.7	1.39	95
FISH #86-20						
BLK				2.6	0.41	25
M	45	43	43	43.7	1.64	100
-7	30	31	23	28.0	1.45	88
-6	34	37	31	34.0	1.53	93
-5	40	43	28	37.0	1.57	96
-4	35	40	33	36.0	1.56	95
-3	11	13	13	12.3	1.09	67
-4	35	47	26	36.0	1.56	95
FISH #86-21A						
BLK				1.5	0.18	10
M	70	78	65	71.0	1.85	100
-7	32	32	37	33.7	1.53	83
-6	31	42	45	39.3	1.59	86
-6	37	53	47	45.7	1.66	90
-4	41	42	50	44,3	1.65	89
-3	24	28	37	29.7	1.47	80
-4	40	33	60	41.0	1.81	87

EOG RESPONSE

	Presentation Series				% LOG
FISH #86-22	1	2	3	MEAN	LOG MEAN AA
BLK				3.5	0.54 43
M	18	19	20	19.0	1.28 100
-7	29	37	38	34.7	1.54 120
-6	35	55	53	47.7	1.68 131
-5	31	41	42	38.0	1.58 124
-4	37	42	50	43.0	1.63 128
-3	27	18	33	26.0	1.41 111
-4	37	50	55	47.3	1.66 131
FISH #86-23					
BLK				13.6	1.13 60
M	80	88	64	77.3	1.89 100
-7	88	112	100	100.0	2.00 106
-6	90	78	72	80.0	1.90 101
-6	85	82	80	84.3	1.93 102
-4	80	88	82	63.3	1.92 102
-3	90	82	72	81.3	1.91 101
-4	83	72	72	76.7	1.88 99
FISH #86-24					
BLK				3.4	0.53 31
M	49	44	62	51.7	1.71 100
-7	50	51	61	54.0	1.73 101
-6	51	52	69	57.3	1.76 103
-s	48	48	80	58.7	1.77 103
-4	54	41	56	50.3	1.70 100
-3	27	33	34	31.3	1.50 87
-4	41	39	56	45,3	1.66 97

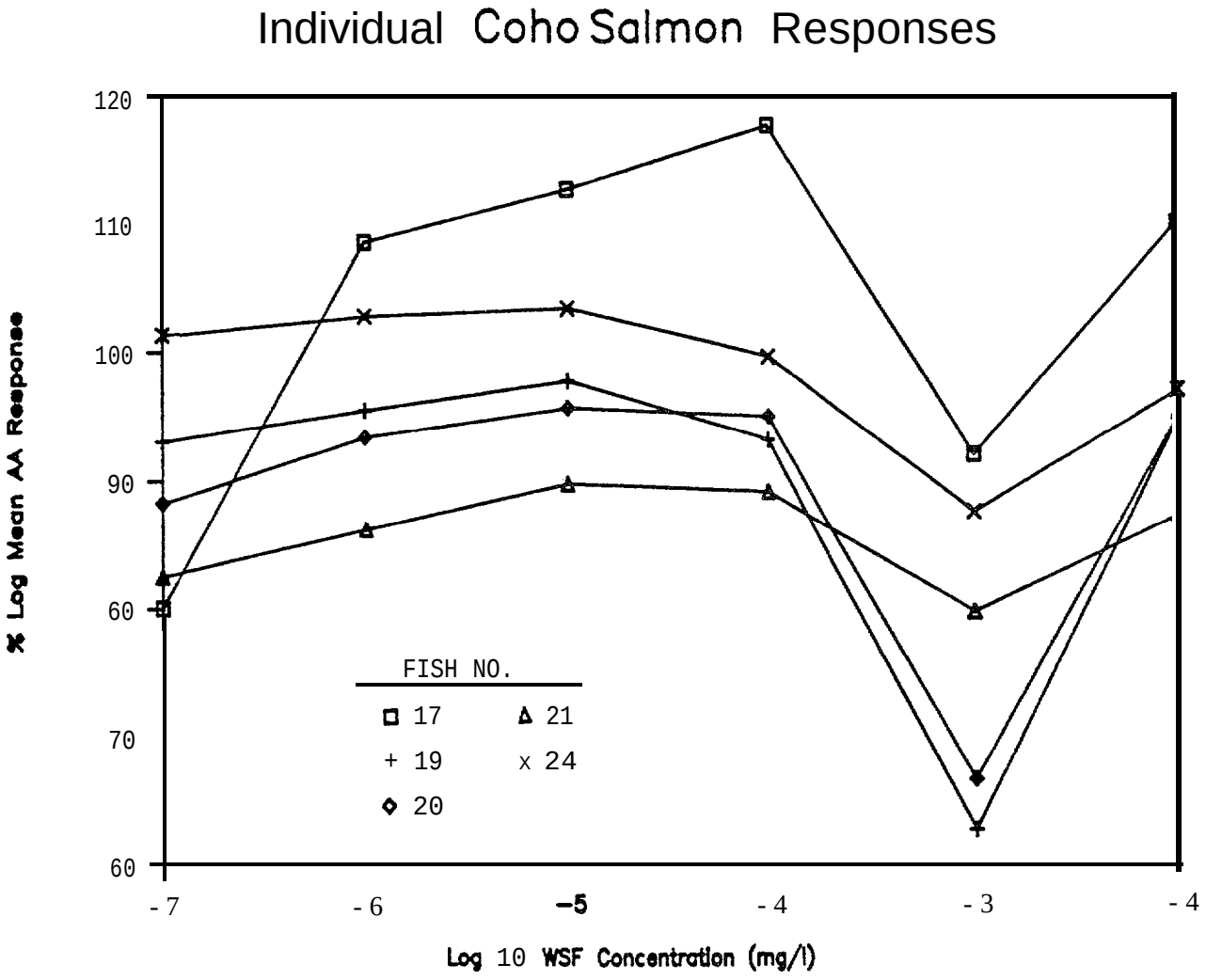

FIGURE 5. The Percentage of the Logarithm of the Mean EOG Response to an Amino Acid Mixture by Five Individual Coho Salmon as a Function of the Logarithm of the WSF Concentrations (mg/l)

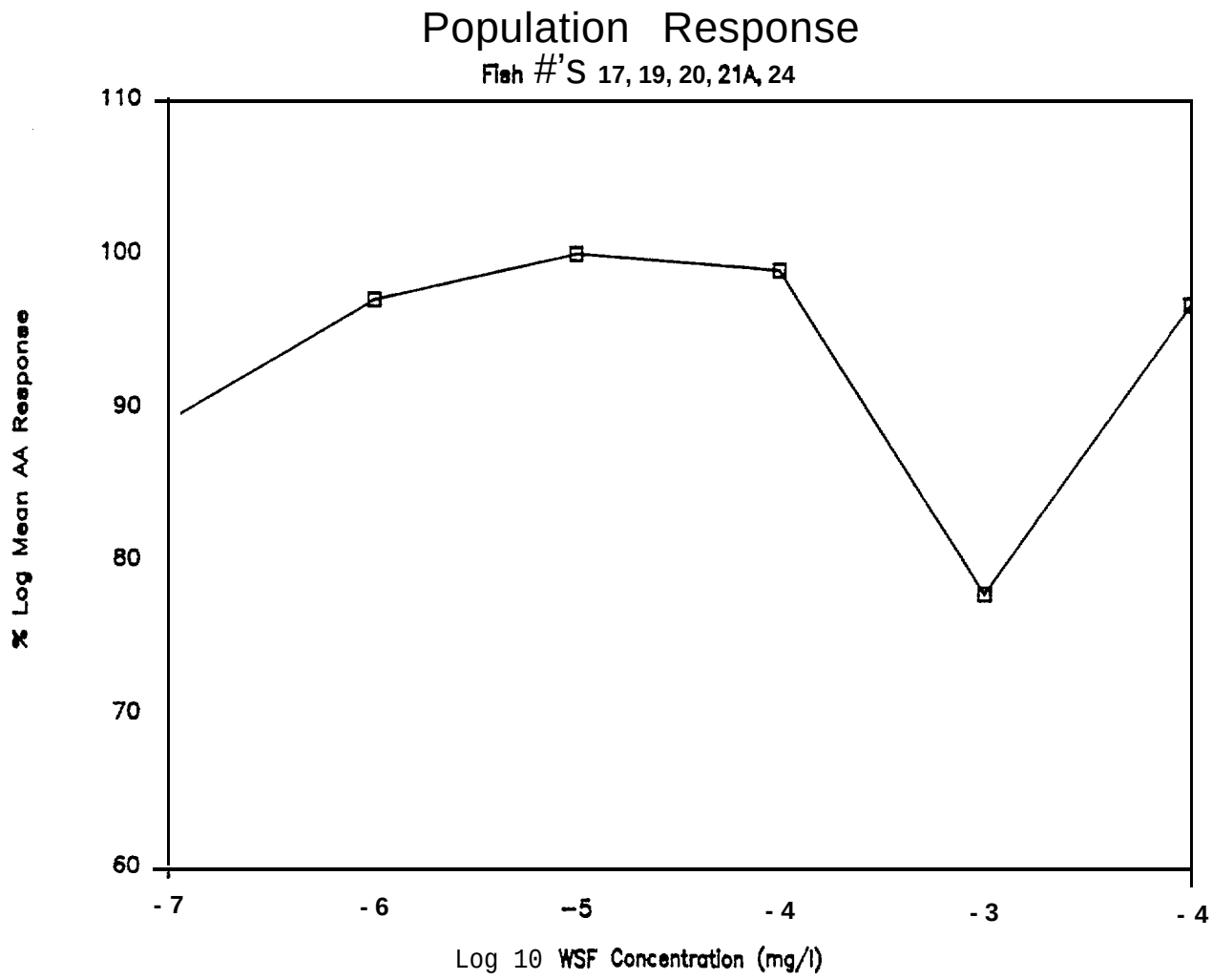

FIGURE 6. The Percentage of the Logarithm of the Mean EOG Response to an Amino Acid Mixture by the Test Population of Coho Salmon as a Function of the Logarithm of the WSF Concentrations (mg/l)

detection thresholds ranging down to 1 X 10‘g M for amino acids. Thresholds for bile acids in salmonids are at least the same and may be somewhat lower (Døving et al. 1980). Thus, the robust responses observed at 10^{-7} WSF would indicate that detection thresholds for WSF are comparable to amino and bile acids. Whether the detection threshold could be appreciably lower is debatable. At dilutions below 11 or 12 log steps, most compounds no longer behave in simple ways, and the creation of uniform dilutions is no longer possible. At these levels, a significant percentage of the stimulus compounds begins to interact with the surface of the glassware. This causes nonuniform stimulus strength and inconsistent responses. Therefore, it is unlikely that thresholds for WSF calculated to be below 10^{-12} mg/l WSF are real . The data, however, indicates a threshold below 10‘grog/l WSF. Fish 86-16 still showed a EOG response above the blank response down to the lowest WSF dilution presented, 10^{-9} mg/l WSF (Table 4), and other coho salmon showed substantial EOG responses at 10^{-7} mg/l WSF (Table 5). Thus, for practical purposes, the detection threshold of WSF by coho salmon may be estimated at 4 x 10^{-10*1} mg/l . This concentration value was derived from the EOG data of Tables 3 and 4 and the chemical data of Table 2 measured by GC and corrected for dilution and loss in the delivery system.

RESPONSES AT HIGHER CONCENTRATIONS

Quite apparent from the response curves presented in Figures 5 and 6 and Table 5 is the degraded response at the 10^{-3} mg/l WSF. This reduction in response is observed in some fish at 10^{-4} mg/l WSF. In fact, the very flat response functions observed for these fish may be caused by an increasing degradation of response as the concentration increases. For other stimuli, there is an exponential increase in response through this range of concentrations (Caprio 1983). A plausible mechanism for our observations involves possible narcotic effects of WSF on cellular elements of the olfactory epitheliums. It has been demonstrated that transport and access of stimuli to receptor sites in salmonids is not through passive circulation. Active transport is required and is achieved by a continuous unidirectional

TABLE 4. Responses in Arbitrary Units of Coho Salmon (Fish 86-16) to WSF and Amino Acid Stimulation.

Log 10 Concentration of WSF	Mean Response	% Log Mean Amino Acid Response
-9	7.0	62
-6	17.0	90
-3	34.0	113
-2	23.0	100
-1	24.0	101
Blank	3.7	43
Amino Acid	23.0	100

TABLE 5. Responses in Arbitrary Units of Coho Salmon to WSF And Amino Acid Stimulation.

		Fish #								
		17	19	20	21A	22	23	24	Pop. Mean	Pop. Mean less fish 22 & 23
Mean Response	BLK	2.2	1.1	2.6	1.5	3.5	13.6	3.4	4.0	2.18
	M	17.0	29.7	43.7	71.0	19.0	77.3	61.7	44.2	42.6
Log 10 concentrations of WSF										
	-7	9.7	23.3	28.0	33.7	34.7	100.0	54.0	40.5	29.7
	-6	21.7	25.3	34.0	39.3	47.7	80.0	57.3	43.6	35.5
	-5	24.3	27.3	37.0	45.7	38.0	84.3	58.7	45.0	38.6
	-4	28.0	23.3	36.0	44.3	43.0	83.3	50.3	44.0	36.4
	-3	13.5	8.3	12.3	29.7	26.0	81.3	31.3	28.9	19.0
	-4	22.5	24.7	36.0	41.0	47.3	75.7	45.3	41.8	33.9
Log Mean Response	BLK	0.34	0.04	0.41	0.18	0.54	1.13	0.53	0.45	0.30
	AA	1.23	1.47	1.54	1.85	1.28	1.89	1.71	1.58	1.58
Log 10 concentrations of WSF										
	-7	0.99	1.37	1.45	1.53	1.54	2.00	1.73	1.51	1.41
	-6	1.34	1.40	1.53	1.59	1.66	1.90	1.78	1.60	1.52
	-5	1.39	1.44	1.57	1.66	1.58	1.93	1.77	1.62	1.56
	-4	1.45	1.37	1.58	1.65	1.63	1.92	1.70	1.61	1.54
	-3	1.13	0.92	1.09	1.47	1.41	1.91	1.50	1.35	1.22
	-4	1.35	1.39	1.56	1.61	1.66	1.86	1.65	1.59	1.51
% Log Mean Ah Resp.	BLK	28	3	25	10	43	60	31	28	19
	M	100	100	100	100	100	100	100	100	100
Log 10 concentrate i ons of WSF										
	-7	80	93	88	33	120	106	101	96	89
	-6	109	96	93	86	131	101	103	103	97
	-5	113	98	96	90	124	102	103	104	100
	-4	116	93	95	89	128	102	100	103	99
	-3	92	63	67	80	111	101	87	86	76
	-4	110	95	95	87	131	99	97	102	97

Mean M responses fo I lowing a '#SF concentrate ion series expressed as % of pre-WSF exposure AA responses

	Fish #						
	17	19	20	21A	22	23	24
% Mean Response	9.9	97.8	106.4	89.0	--- 101.0		104.7

flow propelled by ciliary action (Døving et al. 1977). Therefore, if ciliary activity is inhibited, stimulus access to receptor sites is restricted, fewer stimulus-receptor interactions take place, and the summed response measured by the EOG technique is reduced. The monoaromatic hydrocarbons that comprised 97% of the WSF used here have been implicated as anesthetic or narcotic agents elsewhere (Crisp et al. 1967; Johnson 1977). The rapid recovery following rinsing indicates that no long-term inhibition or damage is caused by short duration presentations of WSF at these concentrations.

EFFECTS OF SHORT-TERM WSF EXPOSURE ON DETECTION OF AMINO ACIDS

To examine the effects of short-term exposure to WSF on the detection of amino acids, the response to amino acid mixtures following stimulation by WSF was measured. Lasting effects from WSF exposure would result in decreased responses to the amino acid stimulation. At all concentrations of WSF tested, 10^{-7} to 10^{-3} mg/l WSF, no change from the preexposure response amplitudes were observed (Bottom of Table 5). An additional observation made during the threshold experiments supports this conclusion. No significant differences among the first, second, or third presentation series were noted. Whereas these exposures are relatively short in duration, approximately 30 see, exposures of 60 to 90 min caused no lasting effects to the responses to amino acid and WSF stimuli in subsequent testing.

DETECTION OF AMINO ACIDS DURING EXPOSURE TO WSF

To determine if salmon could detect meaningful biological stimuli in the presence of WSF, EOG responses to amino acids before, during, and after exposure to WSF were measured. Three presentations of blank, amino acids and 10^{-5} mg/l WSF were presented to the fish in a background flow of APW. Following this series, the background flow was switched to 10^{-5} mg/l WSF. After at least 30 min of exposure, the stimulus series was again presented with the addition of fourth stimulus, an amino acid mixture made in the WSF of background flow. At the conclusion of three presentation series, the

background flow was returned to APW, and the first presentation series of three stimuli was again repeated three times per fish. Responses to amino acid solutions at this concentration of WSF were no different from pre- and postexposure trials (Table 6). These experiments indicate that exposures to 10^{-5} mg/1 WSF for up to 90 min, the duration of the experiment, do not impair detection of amino acids.

EOG RESPONSES AND SPAWNING PRESSURE

A strong relationship between the EOG responses and the level of circulating hormones taken as an index of spawning pressure was not evident. Figure 7 plots the EOG response normalized to the amino acid response for coho salmon presented with 10^{-7} mg/l WSF against the testosterone level of the fish. This EOG response is to a WSF level below those where the responses began to degrade and shows no significant correlation with the testosterone level ($r = 0.627$, $t = 2.06$, $p > 0.05$). Examination of the testosterone levels for the fish in the threshold experiment (Table 1) shows that the hormone levels were within the range of 21 to 44 rig/ml. This range is above levels measured by Hasler and Scholz (1983), who found ranges of 7 to 19 rig/ml testosterone in coho salmon during the open water phase of migration and 12 to 25 rig/ml in ripe males during the upstream phase.

Examination of Figure 5 shows that Fish 86-17 was somewhat different in its EOG responses than the other fish in the threshold experiment. Fish 86-17 was also somewhat different in that it showed an estradiol titer of 0.61 pg/ml whereas all the others had undetectable levels. The data, however, is too sparse to draw any conclusions concerning chemosensory function and spawning pressure as indicated by hormonal levels.

TABLE 6. Mean Responses in Arbitrary Units of Coho Salmon to WSF and AA Stimulation Presented in APW or WSF Background Flow.

	Fish #							Mean
							Population	% of AA
	25	26	27	28	29	31	Mean	response
APW background f low								
BLK	6.6	7.3	6.3	7.0	4.3	6.7	6.3	9.4
AA	42.3	32.0	66.3	72.7	76.7	112.7	67.0	100
WSF 10 -5	16.0	7.0	21.3	10.0	11.7	16.3	13.9	20.7
WSF background f low								
BLK	2.0	6.0	6.0	4.5	0.0	10.6	5.2	7.9
M	62.3	32.5	110.0	75.7	37.7	86.0	65.7	100
WSF 10-6	3.2	20.0	20.8	9.0	4.2	11.7	11.5	1.75
M in WSF 10 -5	---	40.3	161.7	79.7	44.0	99.7	69.2	126.5
APW background f low								
BLK	9.3	5.3	12.3	3.5	4.3	5.0	6.8	9.4
AA	78.0	25.3	74.3	100.0	56.3	91.0	70.8	100
WSF 10 -6	21.0	24.3	27.7	17.7	34.3	13.0	23.0	32.5

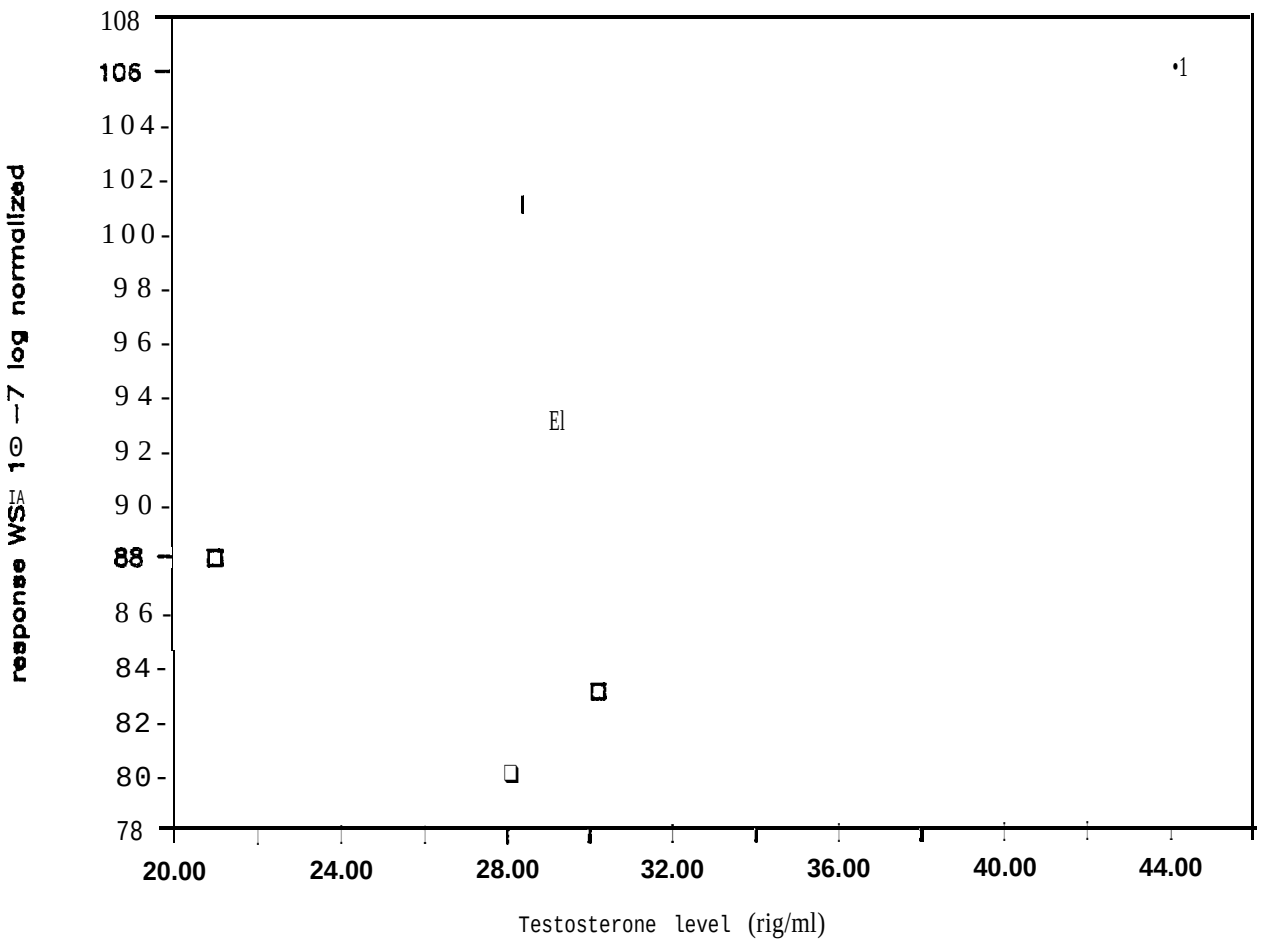

FIGURE 7. The Logarithmically Normalized EOG Response to 10^{-7} mg/l WSF as a Function of the Testosterone Level (rig/ml) in Coho Salmon

DISCUSSION

THRESHOLDS FOR WSF IN SALMON AND OTHER ORGANISMS

The ability of salmon to detect the WSF of crude oil at 10^{-10} mg/l is greater than those reported for other organisms. Hellstrom and Døving (1983) used behavioral criteria to determine that the cod, Gadus morhua, detects the WSF of light diesel fuel at 3 to 30 x 10^{-6} mg/l. Also using behavioral criteria, Dungeness crab, Cancer magister, and blue crab, Callinectes sapidus, were found to detect the WSF of Prudhoe Bay crude oil at 10^{-4} and 10^{-6} mg/l, respectively (Pearson et al. 1980, 1981b).

RELATIONSHIP OF LABORATORY FINDINGS TO OIL SPILL SCENARIOS

The threshold of 10^{-10} mg/l (equivalent to 10^{-7} ppb) found for the detection of WSF by the coho salmon indicates that the fish can detect the presence of hydrocarbons at concentrations 7 to 9 orders of magnitude below levels observed or predicted for accidental oil spills. In accidental spills, the concentrations of hydrocarbons in the water column have varied with the circumstances of the spill. The sum of the observations and estimates discussed in the background section on potential exposure seems to be that maximum hydrocarbon concentrations in the water column can generally be expected to range between 0.2 and 0.65 ppm but can exceed 1.0 ppm where turbulence physically disperses oil into the water column. For spills treated with chemical dispersants, the maximum concentrations appear to be on the order of 20 ppm. Although modelling efforts for Bering Sea oil spill scenarios suggest that the maximum concentrations will not cover large areas or endure long, the modelling effort by Laevastu et al. (1985) predicted that the areas covered by oil concentrations above 1 ppb reach maximums of almost 250 km2 for a tanker accident and 500 km2 for a blowout. Concentrations

above 1 ppb are predicted to continue for about 35 days for the tanker accident and slightly less than 30 days for the blowout.

Based on the above information on the concentrations observed and predicted for oil spills, the implications of our laboratory findings are that in spill situations the salmon are likely to encounter WSF concentrations that the fish can detect over a large area. Also, it s clear that oil concentrations are likely in spill situations that are of the same levels (1 ppb and above) as those found by us to cause decreased chemosensory response to WSF. Because the laboratory tests used a WSF that is predominantly monoaromatic hydrocarbons that are rapidly lost in the weathering of oil slicks, it is not clear whether the salmon can detect weathered oil as well as fresh oil or, more importantly, whether the weathered oil would degrade the chemosensory detection of hydrocarbons. Monoaromatics have been implicated elsewhere as agents of anesthesia or reversible narcosis (Crisp et al. 1967; Johnson 1977) so that their loss through evaporation presumably could eliminate the observed degradation on chemosensory detection of WSF.

While the laboratory findings indicate that the salmon can detect WSF of crude oil at low enough concentrations to avoid a spill, they do not demonstrate that salmon will indeed avoid oil-contaminated water. First, avoidance behavior can be expected to occur at levels several orders of magnitude above the detection threshold. Further, the observed degradation in chemosensory response seen above 10^{-4} mg/l (0.1 ppb) WSF suggests that salmon entering a steep gradient of oil contamination may not avoid it because the fish's ability to detect it may be quickly lost. The circumstances of the spill will determine the gradients of contamination.

The laboratory findings lessen concern that WSF exposure could disorient migrating salmon through impairment of homing cue detection. The laboratory findings provide no evidence that detection of biologically relevant cues was impaired by 90-min exposures of 10^{-5} mg/l WSF and short-term exposures up to

10^{-3} mg/l WSF. The rapid recovery of the EOG response to lower levels of WSF after exposure to higher levels suggests that any effects are reversible. WSF levels above 10^{-3} mg/1 were not tested so that potential disruption of amino acid detection at higher levels and durations cannot yet be discounted. Present evidence confirms our original conception that any such chemosensory impairment would be evident only in the presence of the WSF and therefore would be transient.

PHASE I FINDINGS, FIELD TRACKING STUDIES AND OTHER LABORATORY STUDIES

The findings indicate that the focus of any field tracking studies should be shifted from investigation of potential disorientation by WSF to avoidance of WSF-contaminated areas. The lack of impairment of amino acid detection by WSF up to 10^{-3} mg/l, and the rapid recovery of WSF detection of lower concentrations after exposure to 10^{-3} mg/1 WSF indicates that the likelihood of lasting effects fromWSF exposure on homing cues appears small. The successful return without significant delay of coho salmon exposed to crude oil slicks and dispersed oil by Nakatani et al. (1985) also supports the notion that any chemosensory effects from WSF will occur in its presence and not persist after return to uncontaminated water. In light of these findings, we do not recommend the field tracking of laboratory-exposed salmon as originally proposed.

For Phase II, therefore, we recommend field tracking studies that aim to determine whether migrating coho salmon will avoid areas of the water column contaminated with WSF at concentrations which proved detectable in the laboratory. A target concentration of 10^{-5} mg/1 WSF will be 5 orders of magnitude above the detection threshold and below the point where the degradation of WSF response was observed. For a target concentration of 10^{-5} mg/l, one would need 90 liters or about 22 gallons of WSF at a full-strength concentration of 15 mg/l to cover a portion of the water column 15 m deep by 30 m wide and 300 m long. Such a target concentration is appropriate for testing avoidance because it is 5 orders of magnitude above the detection

threshold, is at a point where the laboratory observations showed no impairment of detection of biological stimuli, and appears feasible from the logistical and permitting viewpoint. To examine avoidance at a target concentration of 10^{-3} mg/l, where the chemosensory response was impaired, would require, for a single fish and the same volume of water, 2200 gallons of WSF. This latter amount appears beyond logistical and permitting feasibility.

There are two remaining questions not covered then by the present laboratory work and the Phase II fieldwork for which we recommend investigation:

(1) Will the salmon avoid oil-contaminated areas with WSF concentrations above 10^{-3} mg/l WSF where the chemosensory response begins to be degraded?

(2) If salmon are exposed to WSF above 10^{-3} mg/l, will the fish become disoriented through impairment of cue detection?

Also, the observed suppression of olfactory responses to WSF in the mid to higher concentrations tested did not allow us to properly calculate a detection threshold. Without knowing the slope of the response function at lower concentrations, we do not know if there is steep decline in response below 10^{-7} mg/l WSF and, thus, a higher threshold than suggested to date. To address these questions, we recommend further laboratory work in two areas:

(1) Evaluation of olfactory response functions at the lower concentrations not fully tested in Phase I and at the higher concentrations above 10^{-4} mg/l WSF where the function appears to degrade

(2) Evaluation of olfactory response to biologically relevant cues under exposure to WSF at levels above 10^{-3} mg/l.

To determine more accurately the nature of the response function, careful measures of responses of stimuli at concentrations lower than 10^{-5} mg/l and higher than 10^{-3} mg/l are needed. To accomplish this important task, particular care must be given to minimizing system contamination so that background noise is reduced. Presentations of stimuli down to 10^{-10} mg/l WSF should be given in an attempt to bracket the threshold and reduce the complications associated with the response suppression observed at higher concentration.

The sharp decrease in responses observed with WSF concentrations greater than or equal to 10^{-4} mg/l WSF must be investigated. At these concentrations, olfactory function may be impaired by continued exposure and even abolished at higher concentrations. To determine if this is the case, WSF concentrations should be presented several log steps higher than those tested to date. Ideally, the highest should correspond to the highest possible concentration that might be encountered in an oil spill (500 ppb).

No impairment of the salmon's ability to detect other biological stimuli has been observed at the concentrations of WSF tested. However, the strong degradation of response to WSF at higher concentrations indicates that this may be possible. To examine this possibility requires an exposure experiment in which the ability of the fish to detect amino acids in the presence of high levels of WSF is measured.

CONCLUSIONS AND RECOMMENDATIONS

Coho salmon can detect remarkably low levels of petroleum contamination. The electrophysiological evidence indicates that coho salmon have an estimated detection threshold for the water-soluble fraction (WSF) of Alaska North Slope crude oil on the order of $10^{-10\pm1}$ mg/l NSF or about 10^{-7} ppb.

At levels of oil contamination orders of magnitude above the estimated detection threshold, the ability of salmon to detect petroleum hydrocarbons is degraded. At WSF concentrations above 10^{-4} mg/l, the chemosensory response to WSFis degraded but not irreversibly. After short presentation of 10^{-3} mg/1 WSF, the ability to detect lower levels of WSF returns within minutes.

For the levels tested, exposure to WSF does not appear to impair the ability of salmon to detect biologically relevant cues. For WSF concentrations from 10^{-7} to 10^{-3} mg/l, short-term exposure to WSF did not result in decreased chemosensory responses to amino acids. Exposures at 10^{-5} mg/l for up to 90 min did not impair amino acid detection.

These findings suggest that coho salmon can detect the presence of dissolved petroleum hydrocarbons at orders of magnitude below the levels seen or predicted to cover large areas during oil spills. The salmon have the sensory ability necessary to avoid oil spills, but field studies are necessary to demonstrate whether migrating salmon will actually avoid oil-contaminated areas.

The implications of the degradation in WSF detection at higher WSF levels for avoidance of oil spills is less clear. Such degradation suggests that where migrating salmon encounter steep gradients to exposure levels

above 10^{-3} mg/l WSF, the fish may have impaired ability to detect and avoid oil-contaminated areas.

The finding of little or no evidence for impairment of biologically relevant cues by WSF up to 10^{-3} mg/l suggests that the salmon can be expected to be able to migrate through these concentrations without becoming disoriented. Levels and durations of WSF exposure above that have not been tested and need investigation.

Based on the laboratory findings of Phase I, we recommend the following:

- Extension of the laboratory studies to include two efforts
  - Evaluation of olfactory response functions at lower concentrations not fully tested in Phase I and at concentrations above 10^{-4} mg/l WSF where the function appears to be degraded
  - Evaluation of olfactory response to biologically relevant cues under exposure to WSF at levels above 10^{-3} mg/l .
- Field tracking studies that concentrate on determining whether salmon avoid WSF concentrations above the detection threshold and below the point at which chemosensory response degrades.

We do not recommend the pursuit of field studies without more laboratory studies. The findings of Phase I shift the focus of Phase II field tracking studies from investigation of potential disorientation of migrating salmon by chemosensory disruption to investigation of avoidance. However, the possibility of disorientation through chemosensory impairment by petroleum remains open, and because of the logistical problems in applying a field treatment of sufficient magnitude to be a valid test, we urge that these be studied in the laboratory. Addressing questions of avoidance and

disorientation above 10^{-3} mg/1 WSF with field tracking appears beyond logistical and permitting feasibility, and both questions can be addressed with laboratory studies. If laboratory studies show that the EOG response to WSF becomes increasingly impaired as WSF concentration raises, then one can reasonably expect that avoidance also becomes increasingly unlikely as its sensory foundation is eroded. Similarly, because we know that migrating salmon that have impaired homing cue detection become disoriented, we can expect such disorientation in the field should laboratory studies indicate that cue detection is impaired above 10^{-3} mg/1 WSF.

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